STUDIES ON FATS AND FATTY ACIDS

(RESUME)

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY
TO THE
ALIGARH MUSLIM UNIVERSITY, ALIGARH

BY
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Aligarh
1980

RESUME

The work presented in this thesis consists of two parts:

Part I concerns with the compositional studies on indigenous seed oils.

Part II deals with the reactions of long-chain internal, terminal, and α,β-unsaturated acids and their derivatives with nitrosyl chloride (NOCl).

PART I

Compositional Studies on Indigenous Seed Oils

The seed oils from twelve species were analyzed for their component acids mainly by chromatographic and spectroscopic techniques. All the seed oils except those of *Mucuna prurita* and *Aesculus indica* were found usual in containing simple type of oleic - linoleic - linolenic acids but in varying proportions.

A. Usual Seed Oils

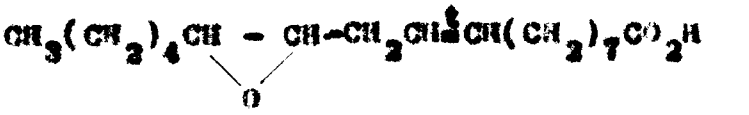
Eight of the ten usual seed oils reported here have not had oil composition reported so far. The gas-liquid chromatography (GLC) analysis confirms that all the oils examined are composed of conventional fatty acids but in widely varying

proportions. The content of the total saturated acids varied from 7.5 - 39.6%. In the combined content of palmitic and stearic acids, palmitic acid was found to be present as a major component in all samples - which is the usual pattern of distribution of palmitic and stearic acids. Other than C_{16} and C_{18} saturated acids, C_{10}, C_{12}, C_{14} and C_{22} saturated acids were also found to be present in various species in minor amounts.

All the species were found to contain high percentage (60.4-92.5%) of unsaturated acids. C_{18}-Unsaturated acids ranged from 60.4-89.1%. A moderately high percentage (27.1) of C_{20}-monoenoic acid (Eicos-11-enoic) was found to be occurring in Delphinium ajacis. Four species namely Centaurea moschata, Grewia flavescens, Jusgia suffruticosa, and Passiflora foetida yielded linoleic-rich seed oils containing 65.4-69.3% of linoleic acid. Linolenic acid was present as a minor constituent (3.9%) in C. moschata only. Fatty acid composition of J. suffruticosa P. foetida, and C. moschata seed oils resembled that of Niger, tobacco and safflower especially grown in tropics. Oils of C. moschata, G. flavescens, J. suffruticosa and P. foetida species containing 65.4-69.3% of linoleic acid with no or little linolenic acid may possibly find used as a linoleic-rich drying oils. A serious consideration can be given to species rich in oils as well as in specific acids if any can meet the agronomic standards of a field crop.

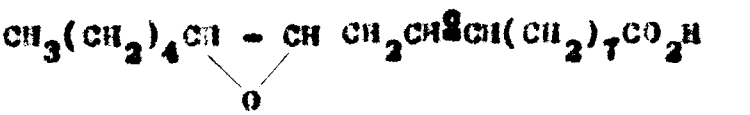
B. A new epoxy acid of Mucuna prurita seed oil

Oil from the seed of M. prurita contains previously unidentified cis-12,13-epoxyoctadec-trans-9-enoic (I, 1%) and the more common vernolic (cis-12,13-epoxyoctadec-cis-9-enoic, II) (4%) acid. Gunstones procedure of direct acetolysis of oil containing epoxy acids in minor amounts was adopted to characterize the epoxy acids in the oil. The techniques used in isolation and identification of the acids included elemental analysis, thin-layer and column chromatography, IR, UV and chemical reactions (viz., reduction and oxidative cleavage) coupled with GLC.



cis-12,13-epoxyoctadec-trans-9-enoic

(I)



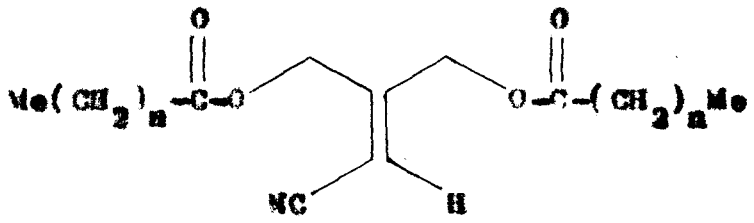
cis-12,13-epoxyoctadec-cis-9-enoic

(II)

C. Cyanolipids in Aesculus indica Seed Oil

Four types of cyanolipids present individually or in pairs have been identified in the seed lipids of the Boraginaceae and Sapindaceae species. More recently the presence of cyanolipids has been shown from our laboratory in Cardiospermum canescens (Sapindaceae), Dodonea viscosa (Sapindaceae) and two species of

Heliotropium (H. indicum and H. eichwaldi). During our compositional studies on indigenous seed oils A. indica seed oil was found to contain one such cyanolipid, namely, the fatty acid diester of 1-cyano-2-hydroxyethylprop-1-ene-3-ol (III, 20%,W/W). Its structure was corroborated on the basis of spectroscopic and chromatographic analyses.



(III)

PART II

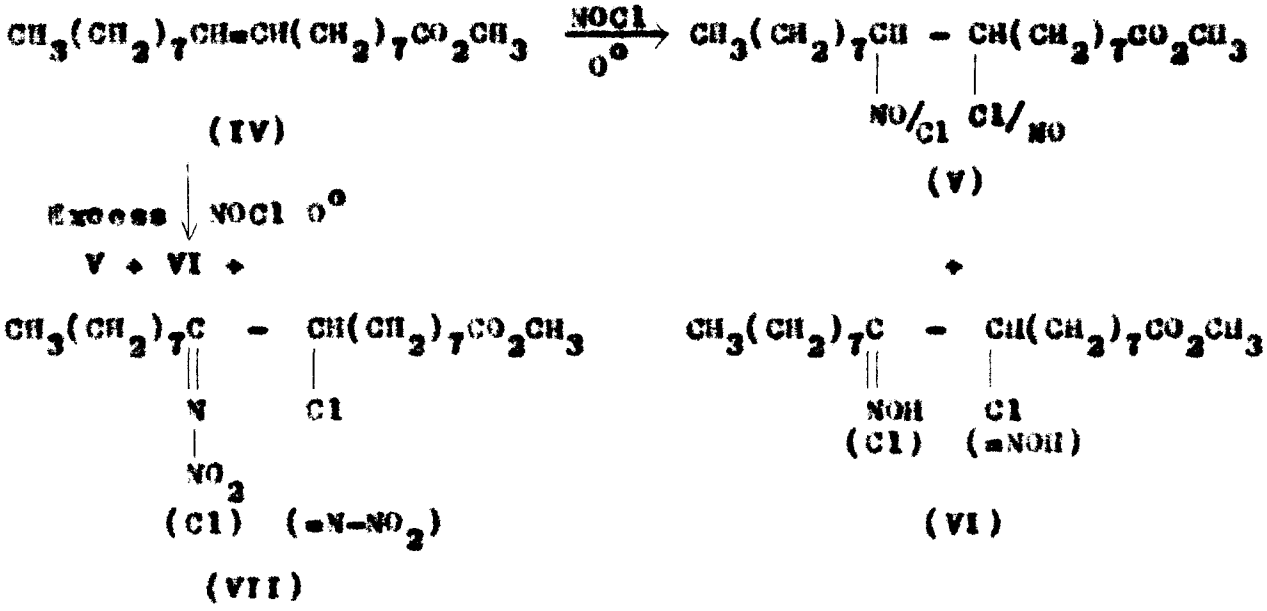
In a continuing study on the reaction of long-chain fatty acids from this laboratory, an attempt was made to study the action of nitrosyl chloride upon internal, terminal, and α,β-unsaturated acids and their derivatives.

A. Nitrosochlorination of methyl oleate

The nitrosochlorination of methyl oleate with approximately stoichiometric quantities of NOCl *in situ* yielded chiefly nitrosochloro product (V, methyl 9(10)-chloro-10(9)-nitrosooctadecanoate) accompanied with some amount of its isomeric oximino form (VI, methyl 9(10)-chloro-10(9)-oximinooctadecanoate). The excess of NOCl yielded an unusual product chloronitrimine (VII, methyl 9(10)-chloro-10(9)-nitriminooctadecanoate) in addition to normal

products (V and VI, Chart I). The formation of chloronitrimine can well be explained through the oxidation of oxime (VI). The structures of products were established by microanalysis, IR and NMR.

Chart I

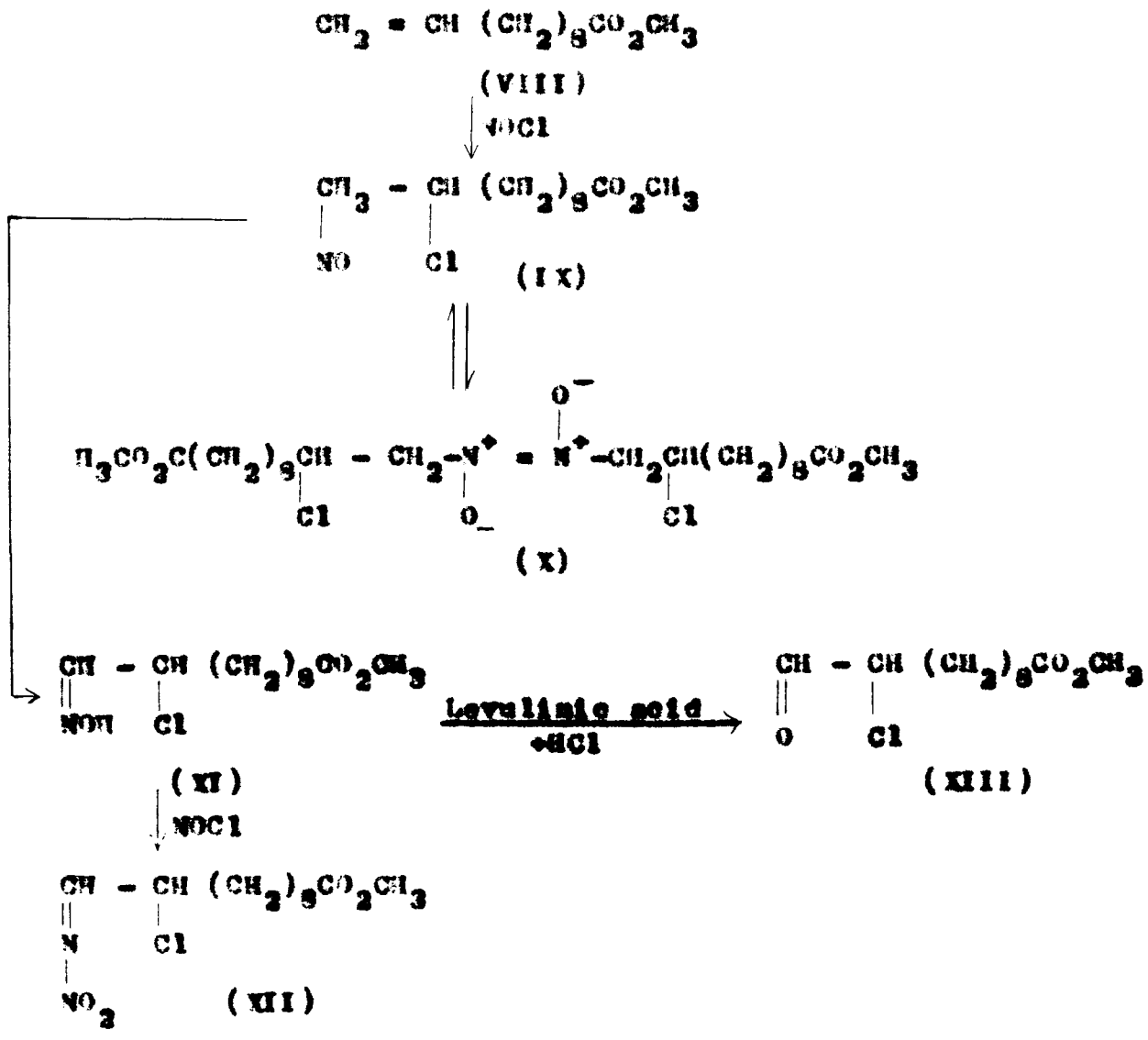


B. Nitrosochlorination of methyl 10-undecenoate

In the present study the methyl ester of 10-undecenoic acid (VIII) was selected as a model substrate for nitrosochlorination reaction for two reasons. Firstly, there appeared to be no mention in the literature of the nitrosochlorination of undecenoic acid. Secondly, it was considered desirable to probe the regioselectivity of nitrosyl chloride addition on an unsymmetrically substituted double bond as well as the steric effect in the formation of product. Reaction of methyl 10-undecenoate (VIII)

with NOCl *in situ*, resulted in the formation of four products (IX - XII, Chart II). Only the components (X - XII) could be isolated and characterized in pure form viz. dimer of methyl 10-chloro-11-nitroso undecanoate (X), methyl 10-chloro-11-oximinoundecanoate (XI), and methyl 10-chloro-11-nitrimino-undecanoate (XII). The compound (IX, methyl 10-chloro-11-nitrosoundecanoate), a primary product, could not be isolated in pure form as it easily dimerizes or rearranges to an oxime.

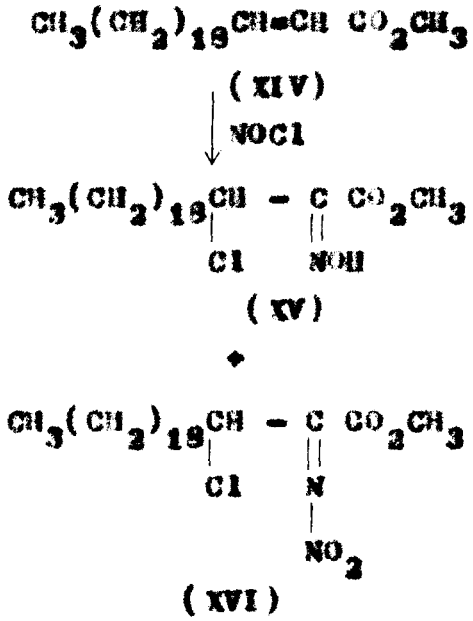
Chart II



C. Nitrosochlorination of docos-*trans*-2-enoate (XIV)

When methyl docos-*trans*-2-enoate was treated with NOCl (*in situ*) at 0-5° for about a month only about 10% of the compound (XIV) was found to undergo nitrosochlorination. The products (XV and XVI, Chart III) were characterized as methyl 2-oximino-3-chlorodocosanoate and methyl 2-nitrimino-3-chlorodocosanoate respectively on the basis of elemental analysis, IR and NMR.

Chart III



D. Reaction of nitrosyl chloride with fatty 1,2-diol (1,2-hexadecanediol, XVII)

The reaction of the vicinal diol (XVII) was carried out with NOCl (*in situ*) in methylene chloride at room temperature. The reaction yielded *iso*-amyl 2-nitritohexadecanoate (XVIII) and *iso*-amyl 2-hydroxyhexadecanoate (XIX). The products were

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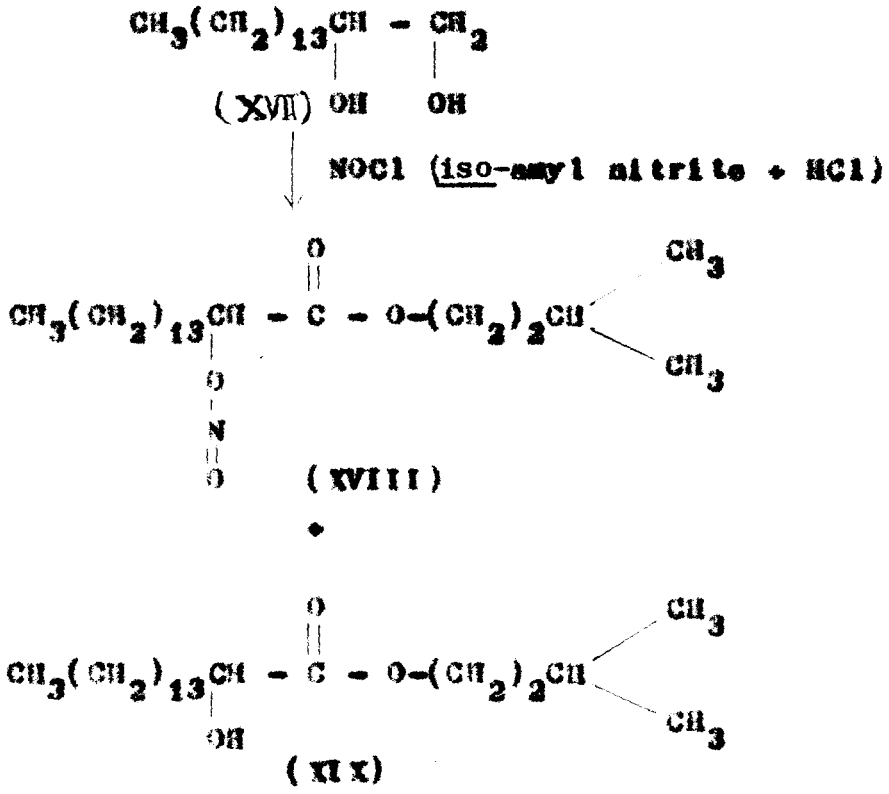
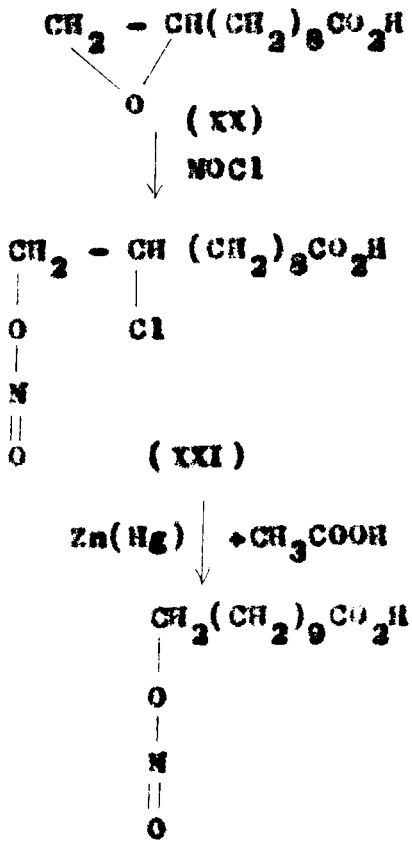
Chart IV



B. Reaction of nitrosyl chloride with 10,11-epoxyundecanoic acid (XX)

The present work on NOCl reaction with epoxide (XX) was undertaken primarily as there appeared to be no mention in the literature on the action of NOCl with oxiranes. Further a terminal epoxide was selected as a substrate to study the direction of ring opening. When 10,11-epoxyundecanoic was treated with NOCl Gas a quantitative yield of 11-nitrito-10-chloroundecanoic acid (XXI) was obtained (Chart V).

Chart V


CH2 - CH(CH2)8CO2H
O
(XX)
NOCl
CH2 - CH (CH2)8CO2H
O
Cl
N
O
(XXI)
Zn(Hg)
+CH3COOH
CH2(CH2)9CO2H
O
N
O

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This is to certify that the work described in this thesis is the original work of the candidate carried out under my supervision. The thesis is suitable for submission for the award of the degree of Doctor of Philosophy in Chemistry.

(S.M. Osman)
Professor of Chemistry

ACKNOWLEDGEMENTS

I wish to express my utmost gratitude to Professor S.M. Osman for his valuable guidance, sincere help and encouragement in carrying out these researches, to Professor Wasiur Rahman, Head, Department of Chemistry for providing the facilities necessary for prosecution of this work.

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I gladly acknowledge the Department of Chemistry, Aligarh Muslim University, Aligarh for awarding the teacher fellowship and University Grants Commission, New Delhi for financial assistance under the faculty improvement programme.

Syed Qamarul Hasan

C O N T E N T S

1. SUMMARY

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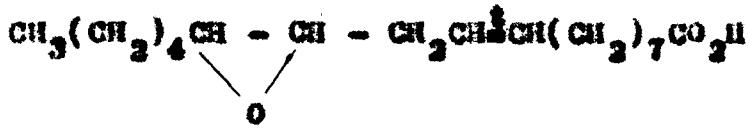
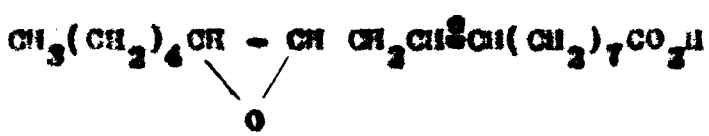


cis-12,13-epoxyoctadec-*trans*-9-enoic

(I)



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(II)

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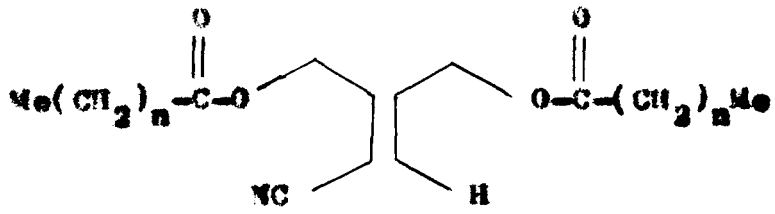

(III)

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In a continuing study on the reaction of long-chain fatty acids from this laboratory, an attempt was made to study the action of nitrosyl chloride upon internal, terminal, and α,β-unsaturated acids and their derivatives.

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The nitrosochlorination of methyl oleate with approximately stoichiometric quantities of NOCl *in situ* yielded chiefly nitrosochloro product (V, methyl 9(10)-chloro-10(9)-nitrosoceta-

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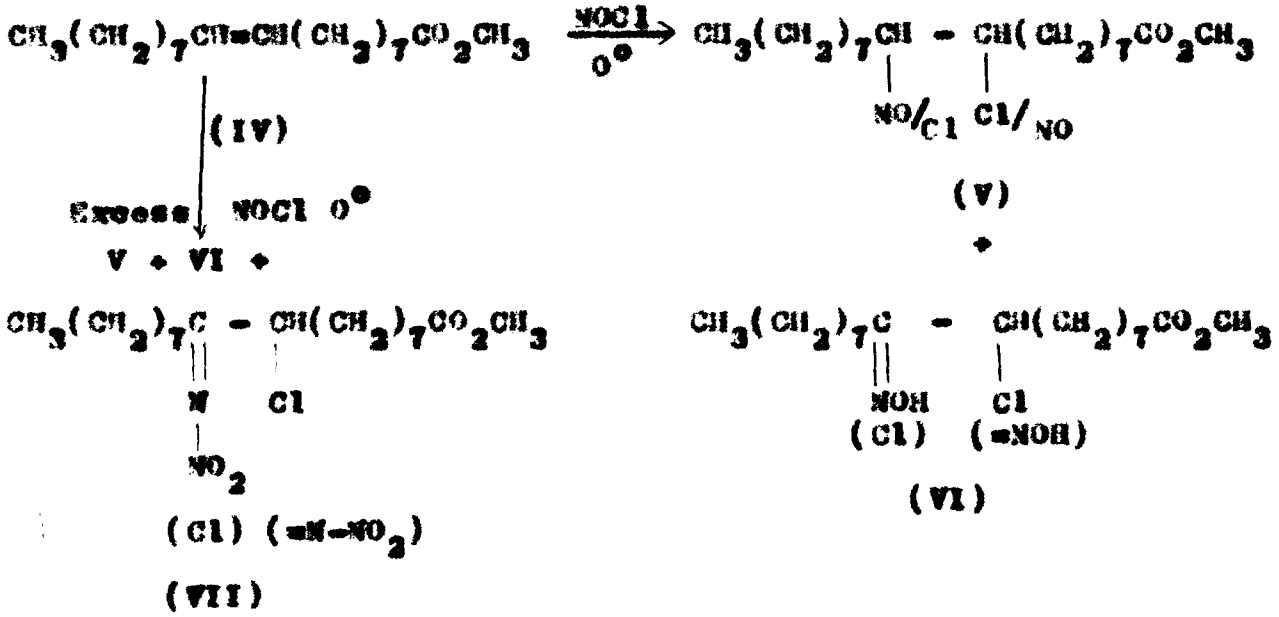


B. Nitrosochlorination of methyl 10-undecenoate

In the present study the methyl ester of 10-undecenoic acid (VIII) was selected as a model substrate for nitrosochlorination reaction for two reasons. Firstly, there appeared to be

no mention in the literature of the nitrosochlorination of undecenoic acid. Secondly, it was considered desirable to probe the regioselectivity of nitrosyl chloride addition on an unsymmetrically substituted double bond as well as the steric effect in the formation of product. Reaction of methyl 10-undecenoate (VIII) with NOCl *in situ*, resulted in the formation of four products (IX - XII, Chart II). Only three components (X - XII) could be isolated and characterized in pure form viz. dimer of methyl 10-chloro-11-nitrosoundecanoate (X), methyl 10-chloro-11-oximinoundecanoate (XI), and methyl 10-chloro-11-nitrimino-undecanoate (XII). The compound (IX, methyl 10-chloro-11-nitrosoundecanoate), a primary product, could not be isolated in pure form as it easily dimerises or rearranges to an oxime.

Chart II

$$CH_2 = CH(CH_2)_8CO_2CH_3$$

(VIII)

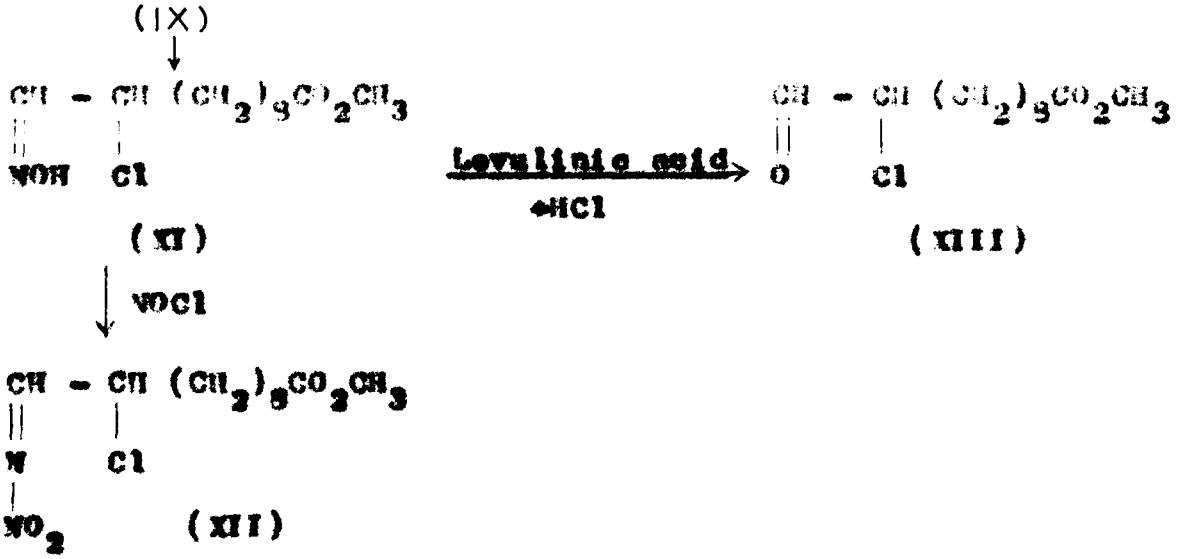
$\downarrow$ NOCl

$$\underset{\underset{NO}{|}}{CH_2} - \underset{\underset{Cl}{|}}{CH}(CH_2)_8CO_2CH_3$$

(IX)

$$H_3CO_2C(CH_2)_8\underset{\underset{Cl}{|}}{CH} - CH_2-\overset{\overset{O^-}{|}}{N^+}=\underset{\underset{O}{|}}{N^+}-CH_2\underset{\underset{Cl}{|}}{CH}(CH_2)_8CO_2CH_3$$

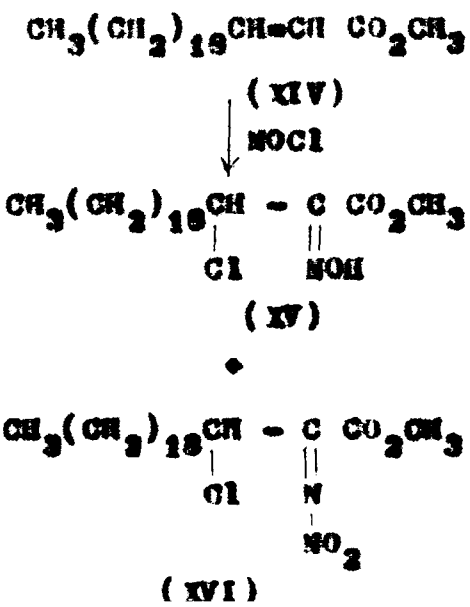
(X)



C. Nitrosochlorination of docos-trans-2-enoate (XIV)

When methyl docos-trans-2-enoate was treated with NOCl (in situ) at 0-5° for about a month only about 10% of the compound (XIV) was found to undergo nitrosochlorination. The products (XV and XVI, Chart III) were characterized as methyl 2-oximino-3-chlorodocosanoate and methyl 2-nitrimino-3-chlorodocosanoate respectively on the basis of elemental analysis, IR and NMR.

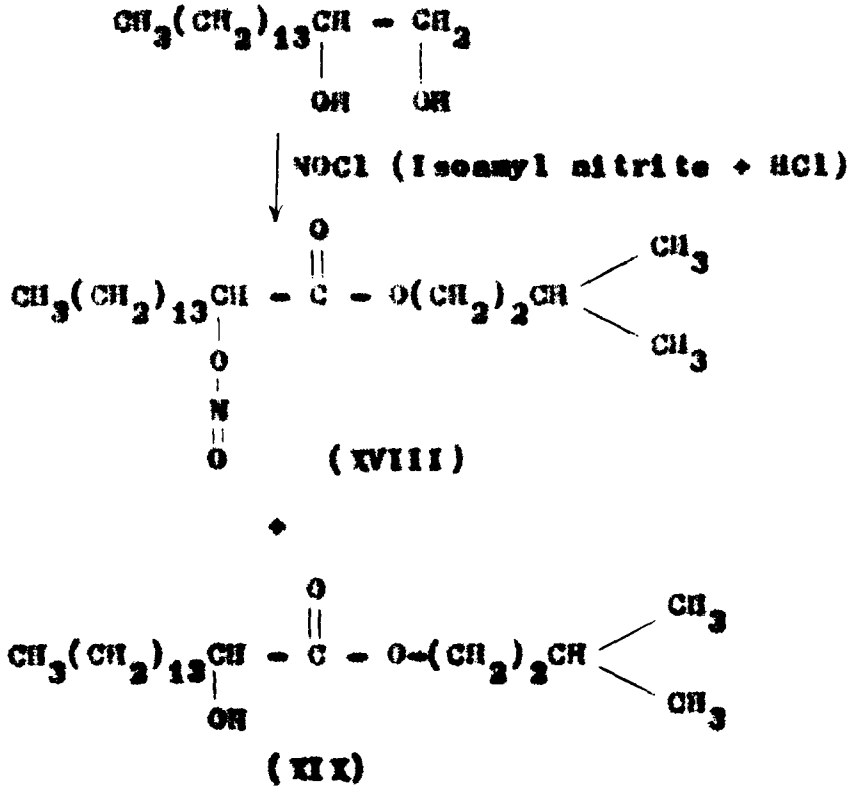
Chart III



D. Reaction of nitrosyl chloride with fatty 1,2-diol (1,2-hexadecanediol, XVII)

The reaction of the vicinal diol (XVII) was carried out with NOCl (*in situ*) in methylene chloride at room temperature. The reaction yielded *iso*-amyl 2-nitritohexadecanoate (XVIII) and *iso*-amyl 2-hydroxyhexadecanoate (XIX). The products were identified on the basis of microanalysis, IR, NMR and Mass. The reaction is depicted in Chart IV.

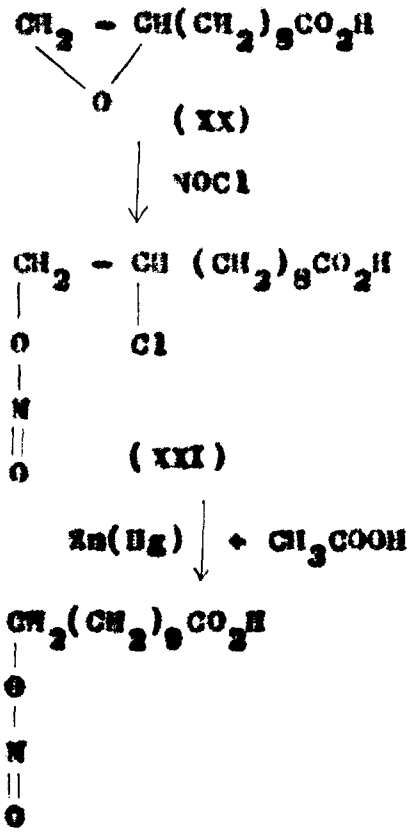
Chart IV



E. Reaction of nitrosyl chloride with 10,11-epoxyundecanoic acid (XX)

The present work on NOCl reaction with epoxide (XX) was undertaken primarily as there appeared to be no mention in the literature on the reaction of NOCl with oxiranes. Further a terminal epoxide was selected as a substrate to study the direction of ring opening. When 10,11-epoxyundecanoic was treated with NOCl gas a quantitative yield of 11-nitrite-10-chloroundecanoic acid (XXI) was obtained (Chart V).

Chart V



2. INTRODUCTION

Although the study of natural products has always been a prominent part of organic chemistry, fatty acids have not been seriously considered in comparison with the more favoured carbohydrates, isoprenoids and alkaloids to mention only a few. Fatty acids generally undergo all classical reactions of organic chemistry. It is rightly pointed out that the different phases of the development and progress of organic chemistry are better exemplified by the general perfection achieved by the chemistry of fatty acid.

The long-chain fatty acids have their origins in land and marine animal fats, vegetable seed oils and organic synthesis. Naturally derived fatty acids are normally considered replenishable in the sense that animals and plants reproduce themselves. There are good reasons for expecting that fatty acids as renewable sources, will become even more important in years to come. There has been a concentration of a study on only two acids oleic and linoleic and their geometric isomers. It has generally been assumed that what is true of oleic acid holds for other monoenoic acids and that what is true for linoleic acid holds for other polyenoic acids. Considering this limited sphere of

investigation in fatty acid chemistry our research activity on fatty acid reactions has been directed towards the reactions of olefinic acids containing terminal, internal, and α,β-unsaturation. Our emphasis has been more on the preparation of new fatty acid derivatives using both classical and non-classical reactions and to study the co-relation of structure and product composition in the preparation of these derivatives. It has been estimated that by the turn of the present century the world population will be doubled and a serious global crisis will arise for the need of oils in the edible and non-edible industries. Consequently the programme of screening uncultivated seed oils has been initiated in many advanced countries with a view to discover new seed oils and fatty acids which may lend themselves to practical utilization.

In early 1960's India used to export oilseeds after meeting its domestic requirements. But now ours is an edible oil deficient country. There is a vast potential of minor oilseeds which if properly trapped, can substantially augment the overall supplies of vegetable oils and help in bridging the wide gap between their demand and supply. Two PL-480 projects on the screening of herbaceous oil-expanding species and synthesis of new fatty acid derivatives have been initiated in the author's laboratory in recent years.

Uncertainty over availability and cost of petrochemicals has rekindled academic interest in natural oils as an alternative raw material source of fatty chemicals. In view of the above objectives the present work described in the thesis deals with the studies on seed oils and the chemistry of fatty acids.

3. PART - I

COMPOSITIONAL STUDIES ON INDIGENOUS SEED OILS

A. THEORETICAL

Over the past ten years the world-wide production and consumption of fats and oils has gone up by 1 to 1½ million tonnes, or some 2% a year. The share of developing countries in the growth has been comparatively small, although some of them such as Malaysia, Brazil, Indonesia and the Ivory cost, have increased the production and export tonnage in recent years. In fact oil occupies a pivotal position for developing country's economy in the present day world.

Our knowledge of natural fats of vegetable origin is very limited to the extent that out of about 2,50,000 known species of plants only 10-12,000 seed oils from 200 families had been analyzed for their fatty acid composition by 1975. Lipid chemists have been actively engaged in basic and applied research aimed at the development of new crops for industrial and edible purposes. The fundamental phase of this type of research is a cooperative screening programme to discover, define and evaluate new or unusual compounds of promising utility in many different directions in plants with a reasonable potential for cultivation. Such screening programme has revealed and continues to indicate seed species whose development into new domestic crops could satisfy

existing needs, or newly developing requirements of our industry as it increases in size and complexity. The species or groups of species, found to have an outstanding potential as new oilseeds, await agronomic improvement through selection and breeding before crop status can be realized.

India is a major oilseed and oil-producing country. No botanico-chemical survey has earlier been carried out in exploring our natural forest resources from wild/semi-wild herbaceous plants and in finding improved crops potential.

Twenty five years ago, lipids were considered to be oily intractable substances that could be separated into simpler components only with great difficulty and they were studied by a comparatively limited number of painstaking researchers. The development of chromatographic techniques, particularly gas-liquid chromatography and thin-layer chromatography, together with advances in spectroscopy, have led to an explosive growth of interest in these compounds and have revolutionized our knowledge of the role that lipids play in the structure and function of cell membranes, as essential dietary components and in numerous biological processes.Keeping in view the line of work described in this thesis an attempt will now be made here to mention briefly the occurrence, detection and analysis of fatty acids in seed oils.

Component fatty acids of natural fats

More than 500 fatty acid structures have now been reported and the following generalization can be drawn from these. Natural fatty acids usually contain an even number of carbon atoms and are most commonly C_{16}, C_{18}, C_{20} or C_{22} compounds; unsaturated acids have double bonds with *cis* or *Z* configuration in certain preferred positions in the carbon chain; polyene acids have methylene-interrupted unsaturation; the acids rarely contain any functional group other than the carboxyl group and olefinic unsaturation. Of course there are exceptions to all these statements. Nevertheless, there are valuable generalizations against which to discuss fatty acid structure. In the last 20 years, new fatty acids possessing structural features quite unusual according to our earlier concept, have been discovered at an unprecedented rate. The unusual structural features of recently discovered natural fatty acids are described in subsequent headings. The structures of these unusual fatty acids of plant origin have been comprehensively reviewed by Smith[1].

Fatty acids containing unusual unsaturation

In recent years among C_{18} acids, mono-unsaturation was found at different positions i.e. 3,5,6 and 11. All *cis*-5,9 and 5,9,12 acids have recently been reported in the seed oils

of Taxus baccata[2] and Larix leptolepis[3] respectively. Very recently ω -5 monoenes, have been found in the seed fat of Grevillea robusta[4]. C_{16} mono-unsaturation in seed fat is not as common as C_{18} mono-unsaturation, though amongst these acids mono-unsaturation has been found at the position, 3,5,6,7 and 9. Spencer and coworkers[5] have reported the presence of 82% hexadec-cis-6-enoic acid in Thunbergia alata seed oil. Recently in our laboratory Zanthoxylum alatum (Rutaceae) seed oil[6] was found to contain hexadec-cis-9-enoic acid to the extent of 15.4% .

Until 1964 only one acetylenic acid, tariric (Octadec-6-ynoic) was known. Since then a number of acetylenic fatty acids have been discovered in the oils of plant families Olacaceae, Compositae, Santalaceae and Simaroubaceae. Poly-unsaturated acetylenic acids have been found in plant seeds from only two families, Olacaceae and Santalaceae. Ligthelm and Schwartz[7] proposed four possible structures for an unknown acetylenic acid in seed oil of Ximenia caffra, and proposed it be named Ximenynic acid. Later, Ligthelm et al. characterized the acid as octadec-trans-11-en -9-ynoic acid[8]. Recently Pearl et al.[9] reported two previously unknown acetylenic acids in the seed oil of Alvaradoa amorphoides (Simaroubaceae). These were characterized as octadec-17-en -6-ynoic and cis-6-ynoic acids.

Previous work[10-12] at Peoria Laboratory has reported the analysis of a number of seed oils of Labiatae and found that the subfamily Stachyoidae is unique in the frequency with which allenic acid occurs. The first allenic acid was reported in Labiatae by Wolff et al.[10] to be (-) octadeca-5,6-dienoic acid (Laballenic). Most recently Osman and coworkers[13] have found the seed oil of Leucas cephalotus (Labiatae) to be the richest source of laballenic acid.

Oxygenated fatty acids

Epoxy acids

Epoxy acids have been reported in seed oils from more than 85 species in about a dozen families. Not counting enantiomeric forms and excluding some additional epoxy acids in cutins, one C_{20} and nine C_{18} cis-epoxy acids have been isolated from seed lipids[14-20]. However, they may be formed in nature, and this remains an unsolved problem, they can be considered as oxidation products of known olefinic acids. The structures can be arranged to show their relationship with the appropriate alkene acids: Oleic acid in the first group, linoleic and crepenynic in the second group and linolenic and other triene acids in the final group. There are five 9,10-epoxides, three 12,13-epoxides and one 15,16-epoxide. Vernolic was the first acid of this class to be discovered by Gunstone in 1954[18] and it is still the most

readily available. Its C_{20} homologue (alchornoic acid) has recently been discovered by Kleiman and coworkers[19] from the seed oil of Alchornea cordifolia (Euphorbiaceae). Recently discovered C_{18} epoxy acids by Spencer[30] in the seed oil of Crepis conyzaefolia (Goun) Dalle Torre (Compositae) are (+)-cis-12,13-epoxyoctadeca-trans-6-cis-9-dienoic (14 %) and cis-12,13-epoxyoctadeca-cis-6-cis-9-dienoic (2 %) acid. A new 3,4-epoxy acid, cis-3,4-epoxyoctadec-cis-11-enoic (17.4 %), has recently been reported from our laboratory in the V. roxburghii seed oil[21].

Hydroxy acids

Natural long-chain hydroxy acids are conveniently divided into three categories. One group has the hydroxyl function at or near the carboxyl or methyl end of the chain whilst those with mid-chain hydroxyl groups can be subdivided into acids with or without conjugated unsaturation. The end-chain hydroxy acids are most likely to have this oxygenated function α or β with respect to the carboxyl group or ω_1 and ω_2 at the methyl end. Such acids are present in lipids derived from brain, wool, seeds, yeast, and cutins. Some α-hydroxy acids occurring in seed oils[22-24] are α-hydroxy derivatives of acids such as oleic, linoleic, linolenic, or sterculic, with which they usually co-occur (e.g. α-hydroxy linolenic[22], D-2-hydroxysterculic acid[23]). It is of further interest that they may also be accompanied by unsaturated acids

with one less carbon atom. For example, Salvia nilotica (Labiatae) seed oil contains oleic, linoleic, and linolenic acids, their C_{17} analogues, and the α-hydroxy C_{18} acids[24]. It is reasonable to conjecture and there is some evidence for this that the α-hydroxy acids are intermediates in a chain-shortening process occurring by α-oxidation. The C_{17} acids have unsaturation in the position expected on this basis; the triene acid, for example, is $\Delta^{9,11,14}$.

C_{18} mid-chain hydroxy acids without conjugated unsaturation appear to be hydration products of oleic, linoleic, or linolenic acid. Hydration of an unsaturated alkene can, of course, yield two hydroxy compounds but the natural process under enzymic contro could well be regiospecific. Prominent among these acids are ricinoleic [D-(+)-12-hydroxyoctadec-cis-9-enoic] acid in castor oil as a major component, and strophanthus (or iso-ricinoleic) an isomer of ricinoleic acid (9-hydroxyoctadec-cis-12-enoic) which was first reported by Gunstone in several Strophanthus oils[25]. In this laboratory ricinoleic acid has been reported as a major component in Hiptage benghalensis seed oil[26] and isoricinoleic (Strophanthus) acid has been found to be the major component of the fatty acids of Wrightia tinctoria,[27] W. tomentosa[27] and W. coccinea[28] seed oils. Higher homologues of ricinoleic and densipolic acids, lesquerolic [(+)-14-hydroxyeicos-cis-11-enoic] and auricolic (14-hydroxyeicosa-cis-11-cis-17-dienoic) acids

have been discovered. Apart from the structural resemblance between these C_{18}/C_{20} pairs, the compounds usually co-exist suggesting that they are biosynthetically related. Further examples of chain extension are to be found among the epoxy and furanoid acids and other cases probably await discovery.

Mid-chain hydroxy acids with conjugated unsaturation can be further categorised into those which contain and those which do not contain acetylenic unsaturation. Smith et al.[26] in 1960 found and characterised 9-hydroxyoctadeca-trans-10,trans-12-dienoic acid, which they named dimorphecolic acid. In the same year Morris et al.[29] Chisholm and Hopkins[30] found independently the mixture of 9-hydroxyoctadeca-10,12-dienoic and 13-hydroxyoctadeca-9,11-dienoic acids in seed oils. These acids were known to be either cis, trans or trans, cis in configuration. Those acids with unsaturation which is entirely olefinic resemble the products of oxidation of polyene acids such as linoleic. By chemical or enzymic reaction, linoleic acid furnishes hydroperoxides at C-9 or C-13 with a double bond shifting into conjugation i.e. 9-hydroperoxy 10t 12c diene and the 13-hydroperoxy 9c 11t diene. The initially formed cis trans dienes pass easily to the trans trans isomers.

Hydroxy acetylenic acids tend to occur in fairly large proportions in some of the species that have acetylenic acids. Ligthelm[31] isolated 9-hydroxyximenynic acid from Ximenia oil.

Recently Miller *et al.*[32] have isolated two new hydroxy acetylenic acids 8-hydroxyoctadeca-10,12-diynoic and 8-hydroxyoctadec-17-en-10,12-diynoic acids from the same seed oil. Powell *et al.*[33] characterised helenynolic acid (9-hydroxyoctadec-*trans*-10-en-12-ynoic), an acetylenic analogue of dimorphecolic acid from *Helichrysum* seed oil. Powell *et al.*[34,35] detected and identified several new hydroxy acetylenic acids in *Agonthegyris* oil, Santalaceae. One of these is the first C_{17} fatty acid (7-hydroxyheptadeca-*trans*-10, *trans*-16-dien-8-ynoic) to be found in quantity in a seed oil[36]. The C_{18} and C_{17} homologues may be related through α-oxidation though the α-hydroxy intermediates have not yet been discovered. It appears that hydroxyacetylenic acid are nearly as numerous as hydroxyolefinic acids in seed oils but may not occur in as many plant families.

There are three modes of occurrence of the long-chain hydroxy acids. Some, like ricinoleic acid in castor oil, form triacylglycerols in the conventional manner so that each glyceride molecule contains only three ester linkages. Others such as Kamala oil[37] and *Lesquerella auriculata* seed oil[38] generate glycerol esters with four ester linkages. Additional bonding results from acylation of the fatty acid hydroxyl group with a further long-chain molecule. The third category contains some of their hydroxy acids in lactone form viz. (s)-13-hydroxyoctadeca-*cis*-9, *trans*-11-dienoic acid lactone (coriolide) from *Mesquina emarginata* seed oil[39]. The lactone rings are of moderate size[39].

Keogh and Zurita[40] isolated a very unusual fatty acid and characterised it as α-(15-hydroxyhexadecyl) itaconic acid from a lichen Ushnea aliphatica. Very recently another new acid was discovered by Keogh and coworker[41] from Usnea meridensis and structure of the acid was established as methyl 3,4-dicarboxy-3-hydroxy-19-oxoeicosanoate acid. Rukmini[42] isolated from Argemone mexicana seed oil a solid acid which was designated as (+)-6-hydroxy-6-methyl-9-oxooctacosanoic acid. However, Gunstone et al.[43] showed it to be a mixture of three oxo acids; 9- and 11-oxooctadosanoic and the oxotriacontanoic in an approximate ratio of 1:2:1. Recently two new dihydroxy fatty acids (non vicinal) have been isolated by Osman et al.[44-45] from seed oil of Peganum harmala and Baliospermum axillare and characterized as 9,14-dihydroxyoctadecanoic acid and 11,13-dihydroxytetracos-trans-9-enoic acid respectively.

Oxo acids

Natural keto or oxo acids are much less common. The keto acids of plant origin are a rather heteregeneous group with no unifying features other than possession of one carbonyl group. Thus far, no fatty acids with more than one ketone function have been discovered. In Oiticica oil the conjugated triene acid,

eleostearic (18:3, 9c 11t 13t), is accompanied by its 4-oxo derivative whilst Chrysobalanus icaco seed oil also contains the conjugated tetraene acid-parinaric (18:4, 9c 11t 13t 15c)- and its 4-oxo derivative[14].

Two oilseeds contain mid-chain oxo acids of longer-than usual chain length. Cuspidaria pterocarpa seed oil contains C_{24}, C_{26} and C_{28} acids[46] all of which have the common C_{10} end-group (I). They could therefore arise from linoleic acid by a selective oxidation at C-9 followed by chain-extension.

$$CH_3(CH_2)_4CH{=}CHCH_2CH_2CO(CH_2)_nCO_2H$$

(I)

n=13, 15 or 17

Argemone mexicana seed oil has been shown by Gunstone et al.[43] to contain three oxo acids with 24 and 30 carbon-atoms (II, III and IV). It is conceivable that these arise biologically from stearic and arachidic acids by a chain-extension process in which

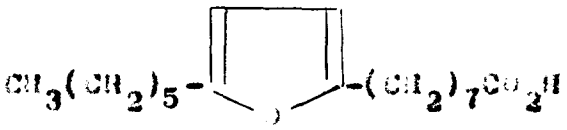
$$CH_3(CH_2)_mCO(CH_2)_nCO_2Me$$

(II) (m=15, n=7)
(III) (m=16, n=9)
(IV) (m=18, n=9)

the original carbon remains in the oxidised form.

Furanoid acids

In the group of natural ether acids which contain a furan ring the first represents an unbranched 18-carbon furanoid acid with an ether linkage between C-9 and C-12 or, alternatively the n-10 and n-7 carbon atoms. This acid (9,10-epoxyoctadeca-9,11-dienoic, V) was first isolated by Morris et al.[47] from a plant source Exocarpus cupressiformis (Santalaceae). It remained a chemical curiosity until the report in 1975 by Glass et al.[48-50] that the lipids of the male Northern Pike (Esox lucius) contain six or more branched-chain furanoid acids. We know little or nothing of the biosynthesis, metabolism, or purpose of these unusual acids.



(V)

There is a growing interest in the oxygenated acids which appear in seed oils after prolonged storage of the seeds. The occurrence of such acids in commercial seed oils makes it important that we know more about their chemistry. Sunflower seed oil, extracted from seeds which have been stored for 2-10 years, contain about 3% of oxygenated acids among which four have been recognized; they include 9,10-epoxy acids related to oleic and linoleic acid and two of the hydroxy diene acids which

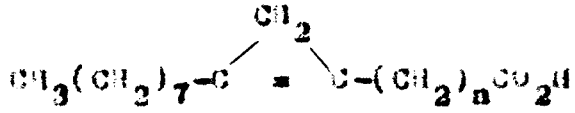
arise, presumably, through oxidation of linoleic acid[51]. The seed oil of Stenochaenium macrocephalum is unusual in containing a rare triene acid with 3t 9c 12c unsaturation. After two years storage of the seeds the extracted oil contains about 6% of epoxy acids and a similar amount of hydroxy acids. Among these are oxygenated derivatives of the unusual triene acid including its 9,10-epoxide and 9- and 13-hydroxy derivatives[15]. Freshly harvested soybeans furnish an oil with about 0.3% of oxygenated acids. After only 1-2 months storage the value has risen to 1-2%[52]. About one half comprises 9,10-epoxy acids derived from oleic, linoleic, and linolenic acids and one third are hydroxy acids including mainly 9,12- and 13-hydroxy C_{18} diene acids with 9- and 12-hydroxy C_{18} monoene acids as minor components.

Alicyclic-substituted acids

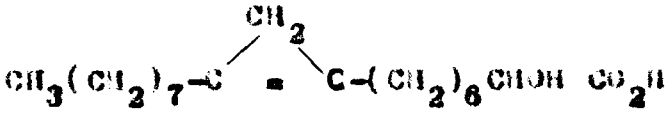
Upto the present three types of alicyclic-substituted acids have been encountered in natural fats, which are (a) Cyclopropane (b) Cyclopropene and (c) Cyclopentenyl (or Cyclopent-2-ene) acids.

Cyclopropane and cyclopropene acids are characterised by the presence of three-membered saturated and unsaturated rings respectively at or near the centre of the hydrocarbon chain. Lactobacillic acid was the first cyclopropane fatty acid to be found in nature, others have now been discovered. The cyclopropane fatty acids are commonly produced by the microorganisms but they

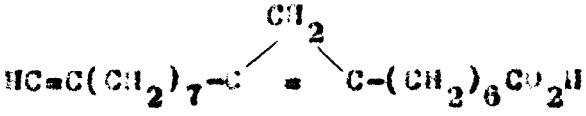
are also found generally in small amounts in certain seed oils where they may be biosynthetic precursors of cyclopropene fatty acids. Dihydrosterculic acid is a major constituent (17.4 %) of the seed oil of Dimocarpus longans (Euphoria longana),[53] Sapindaceae, and it also accompanies the cyclopropene fatty acid, sterculic acid, in many species of the plant order Malvales. Cyclopropene acids have been found principally in seed lipids, though they also exist in other tissues of four plant families of the order Malvales (Sterculaceae, Malvaceae, Bombaceae and Tiliaceae) and may be accompanied by small amounts of the saturated analogue of sterculic acid, dihydrosterculic acid. The sterculic acid was first isolated by Nunn in 1952[54] from Sterculia foetida seed oil and showed it to be 9,10-methylene-octadec-9-enoic acid (VIa). Shortly afterwards malvalic acid (8,9-methyleneheptadec-8-enoic acid, VIb) was isolated and characterized[55-56] and recently two other cyclopropene fatty acids have been discovered, D-2-hydroxysterculic acid[23] (VII) and sterculynic acid (8,9-methyleneoctadec-8-ene-17-ynoic acid,[57] VIII).



(VIa) Sterculic (n=7);
(VIb) Malvalic (n=6)



(VII)

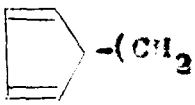


(VIII)

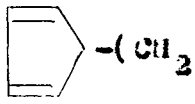
Most recently in our laboratory[59-62] seed oils from Sida acuta, S. rhombifolia, S. grewioides, Hibiscus Sabdariffa Abutilon indicum, Urena lobata, and Eriolaena hookeriana were found to contain sterculic and malvalic acids as unusual constituents of triglycerides in addition to conventional fatty acids.

The fixed oils expressed from the seeds of most of the members of Hydnocarpus genus of the Flacourtiaceae family are commonly known as chaulmoogra oil and are used extensively in the treatment of leprosy and other cutaneous diseases. These oils are characterised by the presence, in predominating amount of unsaturated cyclic fatty acids mainly hydnocarpic, chaulmoogric and gorlic acids, which do not seem to occur in any other seed

fats than those of Flacourtiaceae family. All of these oils contain one or more fatty acids having a terminal cyclopentene ring, especially chaulmoogric (IX), hydnocarpic (X), and gorlic (XI) acids, which account for 80 to 90% or more of the total fatty acids of these oils. Other homologues namely, alepric, alepryllic, alepristic, and aleprolic acids, were found to be present in small proportions in some of these oils.



(IX) (n=12) (X)(n=10)



(XI)

All the evidences of the past years suggest that nature still has some surprise for us in terms of fatty acid structure.

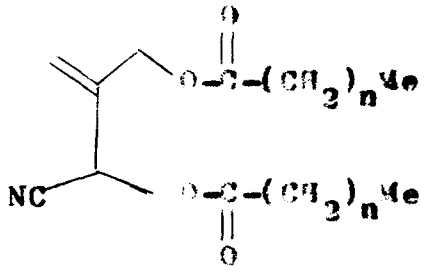
The unusual glycerides and some new classes of Lipids

Glyceride studies have so far been made mainly on fats of industrial or medicinal interest. Our present knowledge about glycerides is fragmentary. Recent discoveries, besides the usual triglycerides, being the common constituents of natural fats, include a variety of typical derivatives of glycerol which have been discovered in seed oils. Several seed oils containing hydroxy acids are known to have more than three fatty acids per glycerol molecule[63]. Recently the oil of Lesquerella auriculata[38] was found unusual in having glycerides containing more than three acyl groups, i.e. more polar tetra acid glycerides. Most recently

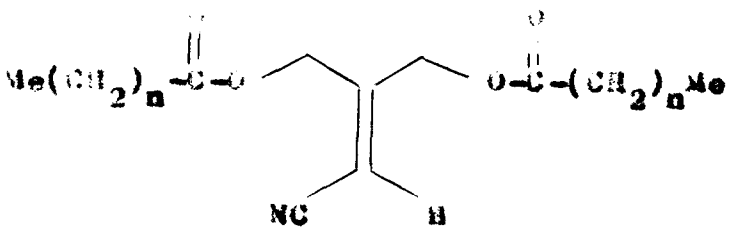
another series of glyceride derivatives have been identified as acetotriglycerides in the seed oil of Monnina emarginata[64] and Celastrus orbiculatus (Celastraceae)[65]. In latter case the monoacetotriglycerides represent 66-98% of the seed oils from selected species in the Celastraceae.

The seeds of Ipomea parasitica[66] contain unique members of a class of glycolipids found in the plant family Convolvulaceae. During the same phase of research the seed lipids of of Rapanea lactevirens (Myrisnaceae)[67] were found to contain a series of 5-alkylresorcinols.

In these, another new class "Cyanolipids" may be added, which co-occur with triglycerides in the seed oils of the families of Sapindaceae and Boraginaceae[68-79]. Cyanolipids were probably first reported in Schleichera trijuga seed oil[80] although the nature of the nitrile moiety was not established at that time. Four types of cyanolipids, present individually or in pairs, have been identified in the seed lipids which are cyanogenetic nonglycerol esters and are derivatives of five-carbon mono- or dihydroxynitrile moiety esterified with long chain fatty acids (XII - XV). Out of these, one class of component is a mixture of diesters containing two fatty acid moieties esterified with 1-cyano-2-hydroxymethylprop-2-ene-1-ol (XII) and 1-cyano-2-hydroxymethylprop-1-ene-3-ol (XIII). The other class of cyanolipids comprise mono- esters of 1-cyano-2-methylprop-1-ene-3-ol (XIV) and 1-cyano-2-methylprop-2-ene-1-ol (XV).



(XII)



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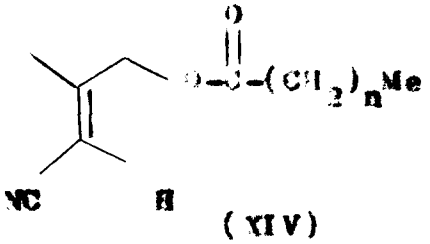

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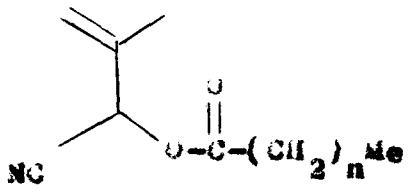


(XV)

Each cyanolipid fraction is a mixture in which the constituents differ only in the attached fatty acids; and because this mixture was difficult to separate and appeared to be based on a single aglycone, it was treated as a single entity during the course of investigations.

Significantly, the hydroxy- or dihydroxynitrile moiety in all four cyanolipids has an isoprenoid skeleton; this permits numerous possibilities for its biogenesis. Since other natural cyano compounds often seem to be derived from amino acids or their precursors[81-83], it should be noted that decarboxylation of L-leucine would give the requisite saturated carbon and nitrogen skeleton for these nitriles.

A curious features of these cyanolipid-containing seed oils is their high content of C20 acids and the preferential

incorporation of these acids into cyanolipids rather than into the accompanying triglycerides. This preference is probably related to the observation that Litchi chinensis seed oil, which has insignificant amounts of C_{20} acids, also contains no cyanolipids[72]. However, the recent work from our laboratory on cyanolipid content of some Sapindaceae[77-79] and Boraginaceae[78] species has revealed that the presence of C_{20} acid is not a pre-requisite for the occurrence of cyanolipids in seed oils as the two species of Heliotropium (Boraginaceae) containing 30-40% of cyanolipids do not contain any C_{20} acid. Additional Sapindaceae and Boraginaceae seed oils that are low in C_{20} acid should be examined for confirmation of this hypothesis.

Both the column chromatographic and preparative TLC procedures used for the isolation of cyanolipids, which are somewhat unstable especially on hydrolysis, are time consuming; therefore Seigler[73] has developed the use of NMR spectra of the chloroform extracted seed oils to determine the presence and amount of cyanolipids and the glycerides with which they occur. All four cyanolipids (XII to XV) may be distinguished from each other and from glycerides by this method.

Isolation and Characterization of fatty acids

A review of the literature indicated that most of the analysis of vegetable seed oils from Indian species were based on the classical methods of oil analysis. Screening analysis of seed oils containing unusual functional groups often gave unexpected responses to analytical procedures in frequent use. Chromatographic and spectroscopic instrumentation have greatly shortened the time needed for organic chemical studies and have increased accuracy in judging homogeneity and purity of materials manifold. The innovations of chromatographic techniques, particularly thin-layer and gas-liquid chromatography, together with advances in spectroscopy, have led to elucidation of many new fatty acid structures and the revision of certain others reported earlier. The main corrections of old mistakes continue to occur in stereochemistry since organic chemists have learnt to determine absolute configurations and preferred conformations by roentgenographic analysis, by NMR spectroscopy and by measurements of circular dichroism.

During recent years a few diagnostic spot tests have been developed for the detection of unusual fatty acids. Some spot reagents include, picric acid for epoxy acids[84]; 4-(p-nitrobenzyl) pyridine for hydroxyl[85] and acetylenic functions[86]; 4-amino-5-hydrazino-1,2,4-triazole-3-thiol for

aldehyde group[87]; 2,4-dinitrophenylhydrazine for keto[88]; ferrous thiocyanate for hydroperoxide[89] and Halphen's spraying reagent for cyclopropenoid acids[23].

The assaying of fatty acids has experienced a total revolution with the development of chromatographic techniques. First developed by the use of adsorption column chromatography,[90] argentation[91-94] and gas-liquid chromatographic techniques were then rapidly adopted for the analysis of fatty acids. The chemical identification of fatty acids by chromatography is based exclusively on the similarity of R_f values or retention times with those of reference compounds of known structure. Unfortunately because of the lack of specificity of these techniques, such criteria are insufficient proof of the chemical identity of a plant product and more specific information such as IR, NMR and MS data are essential requirements for unequivocal establishment of chemical structure.

Modern separative and purification techniques such as chromatography and electrophoresis have greatly facilitated isolation. A phenomenal separation method recently adopted in lipid chemistry is thin-layer chromatography (TLC). Some interesting new approaches involve use of complexing agents during TLC of fatty acids. Impregnation of TLC adsorbents with silver nitrate to aid resolution of cis and trans isomers and higher unsaturates has been suggested by various workers[93-95].

Morris[91,93] also proposed the separation of mono-,di- and poly-hydroxy acid esters. threo and erythro forms of vicinal diols may be separated by making use of boric acid - TLC[95].

In the late fifties, the introduction of GLC along with TLC revolutionized lipid chemistry. Indeed, it is difficult to imagine modern lipid research without these now indispensable tools. GLC now is probably most widely applied method for structural analysis of fatty acids, including polyunsaturated fatty acids (PUFA)[96-99], and is often applied in conjunction with mass spectrometry. Though GLC has brought to the oil chemists a more useful and versatile analytical tool than any which preceded it, several examples will suffice to show some limitations of this technique in application to many types of samples.

In recent years the use of preparative GLC has made it possible the isolation of pure fractions from a complex mixture. The collected fractions can be chemically modified and then re-examined by chromatographic analysis. Keto compounds can be converted to N,N-dimethyl hydrazides, or reduced to hydroxy compounds. Hydroxy esters can be oxidized to keto esters, acetylated or converted to their trimethylsilyl ethers, trifluoroacetyl, or isopropylidene derivatives.

Spectroscopy aids to the recognition and location of functional groups in fatty acid chains. Sometime, the structure can be completely recognised by the use of appropriate spectroscopic techniques with a choice between ultraviolet, infrared, nuclear magnetic resonance (^{1}H NMR with or without chemical shift reagent, and ^{13}C NMR) and mass spectroscopy. On other occasions it is desirable to hydrogenate the acid first, to identify the perhydro derivative and then to tackle the problems related to unsaturation - its nature, configuration, and position.

UV spectroscopy is useful primarily as a means of detecting conjugation in polyunsaturated fatty acids. A direct UV spectrum of an oil showing maxima in the region 200-400 nm gives positive indication for the presence of conjugation. UV determines the number, and to some extent, the kind of multiple bonds in conjugation. As the number of multiple bonds in conjugation increases, UV absorption maxima occur at progressively longer wavelengths.[99-101] For example, dimorphecolic (9-hydroxyoctadeca-*trans*-10,*trans*-12-dienoic) shows absorption maxima in methanol at 231 nm[99], β-eleostearic (octadeca-*trans*-9, *trans*-11, *trans*-13-trienoic) gives $\lambda_{max}^{Ethanol}$ = 259, 268, 279 nm.[99]

In the past, UV has been applied to the structural analysis of PUFA mainly in conjunction with isomerisation procedures. PUFA were analysed by UV after treatment under rigorous alkaline conditions ("alkali isomerisation"). The

number of double bonds in the original acid could be deduced by this procedure provided there was no deviation from methylene-interrupted spacing of double bonds in the starting material. This method is now less frequently used since the advent of GLC.

IR spectroscopy is a considerably more versatile tool for structural analysis than is UV. IR spectroscopy is of particular value in the recognition of unusual functional groups and in detecting and measuring trans unsaturation in fatty acids. One trans bond produces characteristic absorption at 970 cm^{-1}. For non-conjugated polyenoic acids, the effect is roughly additive so that linelaidic acid will have an absorption band at the same position as elaidic acid with increased intensity. Conjugated polyene systems with one more trans bond show a shift in the position of adsorption. Other particular values in the detection of unusual functional groups are 1724 (carbonyl), 3443 (hydroxyl), 1852 and 1010 (cyclopropenoid), 826 and 948 (epoxide), 2222 and 1961 (allene), 952 (conjugated enyne system) and 2240 (nitrile group) cm^{-1}. The use of NMR and mass spectroscopies in the structure determination of fatty acid is described in Part II of the thesis.

Ideally, individual pure fatty acids (usually in the form of the methyl ester derivatives) should be isolated by chromatographic procedures and examined first by non-destructive spectroscopic techniques before chemical degradative procedures

are applied. For example, adsorption chromatography will separate normal fatty acids from those containing polar functional groups. Silver nitrate chromatography can be used to seggregate fatty acids according to the number and geometrical configurations of their double bonds; a portion of each fraction should be hydrogenated so that the lengths of the carbon chain of the components can be confirmed. The position and configuration of double bond(s) may be determined by spectroscopic (principally IR and NMR spectroscopy) and oxidative degradation procedures. Appropriate spectroscopic and chemical techniques must also be used to detect and locate any other functional groups.

Epoxides are indicated by their TLC and GLC behaviour and confirmed by the IR and NMR spectroscopy. The position of an epoxide group can also be determined by mass spectroscopy of the epoxy ester or after conversion to a number of derivatives including the O-methyl, O-trimethylsilyl ether. The readiness with which epoxides undergo cleavage directly with periodic acid or after ring opening to the diol, is the basis of a simple degradation procedure.

The chemical methods are to be employed in almost all the cases for an unambiguous characterization of the fatty acids, inspite of the development of chromatographic and spectroscopic techniques. The chemical methods generally used are hydrogenation, hydroxylation, oxidative degradation, partial hydrogenation and

partial oxidation, hydrogen bromide reaction , addition of dienophiles like maleic anhydride (Diels-Alder reaction). Besides these reactions some specific procedures have been found to be more useful for solving special types of structural problems. These include HBr titration of oil before and after reduction by $LiAlH_4$, cleavage of saturated hydroxy acid by solid $KMnO_4$, dehydration of a dienol to all trans triene acids by treatment with glacial acetic acid, reduction of secondary alcoholic groups $-CHOH-$ to $-CH_2-$ by HI and P, reductive removal of hydroxyl group by the reduction of the tosylate with $LiAlH_4$ followed by oxidative degradation of unsaturated acid by permanganate periodate and lipoxidase catalyzed isomerization to conjugated acids.

B. PRESENT WORK

Fatty acid analysis of indigenous seed oils

Fifteen years ago, our knowledge of the composition, structure and biochemistry of lipids was meagre indeed. The recent major developments have been due mainly to the availability of new analytical and chromatographic techniques. A survey of the literature on seed oil composition indicates that most of the oils analyzed earlier by classical methods are now found to contain less familiar acids possessing a variety of function groups. The older methods of studying fatty acid composition of oils were inadequate to detect very minor components of fatty acid.

Vegetable oils constitute an important part of human diet apart from their various industrial applications. Demand for these oils has been rapidly increasing with improvement in general standard of living, increase in population and technological advances but the supply has not increased in the same proportion thus creating increasing gap. It is well-known that we are an edible oil deficient country. The per capita availability of oils and fats is only 3.2 kg compared to 22 kg recommended on nutritional grounds. From being an oil exporting country in 1966, India today has become seriously dependent on edible oil imports. The acute scarcity and rising prices of

vegetable oils for edible purposes and industrial use had stimulated research in the screening of oil-bearing seeds from wild plants for finding nontraditional sources of vegetable oils. It is now realized that systematic screening of indigenous seed oils may discover oils containing either a high concentration of one of the common natural fatty acids or less common or unknown acid having a structure of scientific interest.

With this viewpoint a preliminary study of a wide range of seed oils, derived mainly from tropical areas, was undertaken at author's laboratory in the hope of finding some of commercial value. As a byproduct of this activity it was expected that fatty acids of novel structure which could be of academic interest having practical value also, might be discovered. A wide range[13,21,26-28,44,45,58-62,77-79,102-117] of seed oils derived mainly from seeds of herbaceous and wild plants have been investigated for their component fatty acids. Prominent amongst these are oils of Hiptage benghalensis[26]; Wrightia tinctoria[27], W. tomentosa[27], and W. coccinea[28]; Leucas cephalotus[13]; Sida species[58]; Peganum harmala[44] and Daliospermum axillare;[45] and Vernonia roxburghii[21] which were found to contain ricinoleic, isoricinoleic, allenic, sterculic and malvalic, new dihydroxy, and a new epoxy fatty acid respectively as their unusual fatty components in major or minor amounts. Some Sapindaceae[77,79] and Boraginaceae[78] species were found to contain a non-glycerol

cyanogenetic lipid in the seed oils. In continuation of this programme the present work describes the seed properties, oil characteristics, and the fatty acid composition of few seed oils belonging to different botanical families.

The initial analysis of oils from all samples showed no significant amount of unusual components. GLC confirmed that these oils are composed of common fatty acids but in varying proportions. The seed analyses and oil properties are given in Table I and the fatty acid composition (wt.%) is recorded in Table II. Oil content of the seeds (Table I) was quite variable and ranged from 1.5% for Grewia flavescens to 32.0% for Delphinium ajacis. Iodine values of the oils ranged from 32.8-145.0.

Seed oils from ten species belonging to nine botanical families have been analyzed for their fatty acid composition (Table II) using chromatographic and spectroscopic techniques. The possibility for the presence of unusual characters like conjugated polyunsaturation and trans unsaturation was ruled out with the help of UV and IR analyses. Various TLC techniques revealed the absence of oxygenated and/or other unusual functional group. Esters gave clear spots on argentation TLC[11,16] corresponding to the saturates, monoene, diene and triene (item 8, Table II) parallel to those from authentic linseed ester resolved alongside. Presence of C_{20} monoene was detected in one seed oil (item 5, Table II). The presence of C_{16} and C_{18}, saturated acids in all

the esters along with C_{10}, C_{12}, C_{14} and C_{22} saturated acids in some species was established with the help of reversed-phase TLC[119]. The quantitation of fatty acids as their methyl esters was carried out by GLC analysis using both polar and non-polar columns (DEGS, 15% and SE-30, 2%) and by measuring the peak area by integral method. Identification of each acid was made by comparing its retention time with that of a standard sample run under the same conditions. The results of the quantitative, direct, reversed-phase and argentation TLC supported the findings of GLC analyses.

The total content of saturated acids varied from 7.5 - 39.6%. All the oils contained palmitic and stearic acids, combined content ranging 7.5 - 30.9%. Seed oils of Cyperus exaltatus (item 4, Table II) and Sesbania sesban (item 10, Table II) were found to contain palmitic acid as high as 22.1 and 26.1% respectively. The stearic acid was present to the maximum of 7.6% in Grewia flavescens (item 6, Table II). In the combined content of palmitic and stearic acids, palmitic acid was found to be present as a major component in all samples which is the usual pattern of distribution of palmitic and stearic acids. Other than C_{16} and C_{18} saturated acids, C_{10}, C_{12}, C_{14} and C_{22} saturated acids were also found to be present in various species in minor amounts. The C14:0 acid (myristic) was comparatively more frequent, found in five species examined (items 2, 4, 7, 8 and 10, Table II). C22:0 acid (behenic) was found to be

occurring in one species only (item 4, Table II) to the extent of 4.7%. Seed oil of S. sesban contained the maximum amount of saturated acids (39.6%).

The total of unsaturated acids in the species examined varied from 60.4 - 92.5%. C_{18}-unsaturated acids ranged from 60.4-89.1%. Among the C_{18}-unsaturated acids, oleic and linoleic acids were found to be the most frequently occurring acids rather than linolenic acid which was present as minor constituent (3.0%)in one species only (item 3, Table II). The combined content of oleic and linoleic acids was found to vary in the region 60.4 - 93.2% in all the ten seed oils. Centaurea moschata and Passiflora foetida contained 92.6 and 83.2% of oleic-linoleic acids respectively. A moderately high percentage (27.1) of C_{20} monoenoic acid (Eicos-11-enoic) was found to be occurring in D. ajacis (item 3, Table II). Four species namely C. moschata, G. flavscens, Jussia suffruticosa and P. foetida yielded linoleic-rich seed oils containing 65.4-69.3% of linoleic acid. Bauhinia malabarica also contained a prominent amount of linoleic acid (37.1%). In species Celosia argentea, C. exaltalus, Ipomoea petaloidea and S. sesban the linoleic acid was only of the order of about 33 ± 5 per cent accompanied by almost similar amount of oleic acid (23.9-37.1%). In D. ajacis linoleic acid falls to 18.9% of the total fatty acids and monoenoic acids (oleic and eicos-cis-11-enoic) take its place as the predominating components (73.6 %oleic, 46.3; and eicos-cis-11-enoic, 27.1%).

Eight of the ten species reported here have not had oil composition reported so far. Three species (C. moschata, D. ajacis and P. foetida) deserve agronomic evaluation as the P. foetida and C. moschata resembled Safflower (especially grown in tropics) and the D. ajacis yielded oleic-rich (46.5%) seed oil accompanied by eicos-cis-11-enoic acid in major proportion (27.1%). The eicos-cis-11-enoic acid content in D. ajacis was found to be maximum among Delphinium species reported so far[120]. The characteristics, but not the fatty acid composition, of P. foetida seed oil have been reported by previous workers[121-122] which are similar to our results. The fatty acid composition reported in P. edulis by earlier workers[123-124] shows that it is linoleic-rich (73-73.7%). The seed oil of P. foetida (item 5, Table II) reported here was also found to be linoleic-rich(67.4%). Most of the oils belonging to Onagraceae family are reported to be linoleic-rich. The present investigation on J. suffruticosa (item 8, Table II) seed oil, belonging to family Onagraceae, also revealed the presence of linoleic acid as a major component (69.3%) but no linolenic acid. Fatty acid composition of J. suffruticosa, P. foetida, and C. moschata seed oils resembled that of Niger, tobacco and safflower especially grown in tropics. A large number of Ipomea seed oils have been analyzed by Barclay and Earle[122]. Some of the species showed the presence of hydroxy acid. Gunstone et al.[125] reported that 18:2 > 18:1 > 18:3 with significant amount of 16:1 and 20:0 acids in I. nadeaceae oil.

The seed oil of I. petaloidea (item 7, Table II) studied here was also found usual and confirmed the earlier general sequence of the composition of Ipomea (18:2 > 18:1) but did not account for 16:1, 20:0 and 18:3 as well. The fatty acid composition of S. sesban, showing palmitic, oleic, and linoleic acids as major components, was found almost similar to other previously reported species of Sesbania genus[126,127] the only difference being in the palmitic acid content which was found to be fairly higher than in species reported earlier[126,127]. The fatty acid compositional studies on C. argentea seed oil (item 3, Table II) conducted by the earlier workers[128] with the help of GLC shows a wide variance from our results. The previous report shows the presence of 16:0, 18:0 and 18:1 as major and 14:0 and 16:1 as minor component acids with the complete absence of linoleic acid, the total saturated content being 70.4%. However, our results showed the prominent amount of linoleic acid (38.4%) and the total of saturated acids approaching only 24.7%. Compositional variation within the species might be due to climatic conditions. The observation made earlier that a given plant species, capable of existence in different climates, produces when grown in a cold climate more unsaturated (linoleic and linolenic) acids in its seed fat than when it is grown in a warmer climate is supported by a comparison of the fatty acid composition in C. argentea species procured from two different climates. The oils from a number of Bauhinia species have been studied for their fatty

acid composition by earlier workers,[124, 125, 129-132] but no work on B. malabarica seed oil seems to have been reported so far. A comparison with previously reported species shows that oil from B. malabarica is almost similar in fatty acid composition to earlier reported species - containing predominently linoleic acid with no linolenic acid. Seed fat of family Cyperaceae is known to contain only usual fatty acids. Oil from C. exaltatus (tem 4, Table II), unworked so far, was also found to be usual like other Cyperaceous species.

Oils from B. malabarica and I. petaloidea which contain 42.2 and 57.1% of linoleic acid respectively with no linolenic acid may be grouped as 'Semi-drying' oils, whilst C. argentea C. exaltatus, B. elegia, and S. sesban which contain less of linoleic acid (below 40%) and no linolenic acid belonged to the class of "non-drying" oils. C. moschata, G. flavescens, I. suffruticosa and P. foetida seed oils may be classified as "drying" oils - containing 63.4 - 70.1% linoleic and linolenic acid. As the linolenic acid is not an essential ingredient of a "drying" oil so long as sufficient linoleic acid (66% or more of the total fatty acids) is present, the oils from above mentioned species containing 63.4 - 69.3% of linoleic acid with no or little linolenic acid may possibly find use as a linoleic-rich drying oils. A serious consideration can be given to species if any can meet the agronomic standards of a field crop.

EXPERIMENTAL PROCEDURES

(i) Source of oilseeds: The seeds used as sources of oils, for the present study, were procured from various sources, including established commercial suppliers, and mostly from collections of wild plants especially grown in the states of U.P. and Rajasthan.

(ii) Oil extractions: Cleaned, dry samples of seeds were usually ground in a disintigrator. The powdered seeds were extracted exhaustively with petroleum ether (bp 40-60°) in a Soxhlet apparatus until no more oil was available. The solvent was removed at reduced pressure under nitrogen to find out the oil content of the seeds. The crude oil was neutralized by passing it (~1 g) in chloroform solution, through a short column of alumina (~10 g). Seed and oil properties viz. moisture content, iodine value, saponification value, refractive index determined by AOCS methods[133]. Nitrogen (crude protein) of the defatted seeds was also determined by the AOCS procedure[133].

(iii) Preparation of mixed fatty acids: Seed oil was refluxed with ethanolic potassium hydroxide. The unsaponifiable matter was removed and the free fatty acids were obtained in the usual manner. Wherever necessary, saponification was carried out under

TABLE I

Source	Seed Analysis			Oil Properties		
	Oil content %	Protein content Nx6.25%	Moisture	I.V.[a]	S.V.[b]	Ref. index n_D^{29}
1. Bauhinia malabarica (Leguminosae)	4.0	19.0	8.5	120.7	202.3	1.4715
2. Celosia argentea (Amaranthaceae)	7.5	20.0	9.0	104.0	202.0	1.4520
3. Centaurea moschata (Compositae)	20.1	18.0	9.2	145.0	193.0	1.4870
4. Cyperus exaltatus (Cyperaceae)	8.7	26.0	9.6	82.8	199.4	1.4630
5. Delphinium ajacis (Ranunculaceae)	32.0	20.2	8.5	98.0	185.1	1.4770
6. Grewia flavescens (Tiliaceae)	1.5	9.0	11.0	124.2	190.2	1.4610
7. Ipomoea petaloidea (Convolvulaceae)	10.3	23.7	7.5	108.9	201.7	1.4790
8. Fuchsia suffruticosa (Onagraceae)	16.2	21.9	8.3	133.7	192.6	1.4875
9. Passiflora foetida (Passifloraceae)	21.2	25.6	8.7	132.2	190.3	1.4850
10. Sesbania sesban (Leguminosae)	7.8	24.4	7.0	86.0	205.0	1.4835

a = Iodine value b = Saponification value

TABLE II

Source	Methyl ester composition, % by GLC						
	14:0	16:0	18:0	18:1	18:2	18:3	Others
1. *Bauhinia malabarica*	-	19.6	3.4	19.9	37.1	-	-
2. *Celosia argentea*	3.7	13.9	4.5	36.9	38.4	-	10:0(0.5) 12:0(2.1)
3. *Centaurea moschata*	-	9.4	1.5	19.0	66.2	3.9	-
4. *Cyperus exaltatus*	4.4	22.1	6.1	32.2	30.5	-	22:0(4.7)
5. *Delphinium ajacis*	-	6.5	1.0	46.5	18.9	-	20:1(27.1)
6. *Grewia flavescens*	-	12.4	7.6	11.2	68.8	-	-
7. *Ipomoea petaloidea*	1.5	15.2	4.0	37.1	42.2	-	-
8. *Jussia suffruticosa*	5.0	13.3	2.4	10.0	69.3	-	-
9. *Passiflora foetida*	-	13.3	4.1	17.2	65.4	-	-
10. *Sesbania sesban*	9.7	26.1	4.8	23.9	36.5	-	-

nitrogen and samples were stored at low temperature in a nitrogen atmosphere.

(iv) Preparation of methyl esters: Esterifications were carried out as follows, except where specified. Fatty acid samples were refluxed for 1 hr in a large excess of absolute methanol containing 1% sulphuric acid (V/V). In each case, resulting mixtures were diluted to the cloud point with water, chilled in an ice bath, and then extracted repeatedly with ether. Combined extracts were dried over sodium sulphate, and filtered and the solvent was removed with the aid of a water aspirator.

(v) Thin-layer chromatography: TLC analyses of the oil as well as the methyl esters were done on plates coated with 0.25 mm or 1.0 mm thick layers of Silica Gel G or 20% silver nitrate-impregnated Silica Gel G with 20% or 30% ether in hexane as the developing solvent. For reversed-phase TLC, the dried, coated plates were uniformly impregnated with Silicone oil (E. Merck). Solvent system acetonitrile-acetic acid-water (70:10:20; V/V/V) was used for development. Layers of Silica Gel G 1 mm thick and hexane-ether (85:15; V/V) were used for preparative TLC of the esters. After preparative plates were sprayed with 2',7'-dichlorofluorescein, bands were visualised under ultraviolet light; then the separated components were recovered by the usual procedure. The spots on all analytical TLC plates were visualised

by charring the plates at 120° after they had been sprayed with a saturated solution of chromium trioxide in 50% aqueous sulphuric acid.

(vi) Gas-liquid chromatography: GLC analyses of methyl esters were carried out as described by Miwa[134]. A Perkin-Elmer Model 154, equipped with thermal conductivity detector, using stainless steel packed column (2m x 3 mm) coated with diethyleneglycol succinate (DEGS, 15% as chromosorb, W, 45-60 mesh) and a 60 cm x4mm column of Silicone (SE-30, 2%). Temperature at the injection port, detector block, and column were 290°, 280° and 190° respectively. Attenuation 4, bridge current 150 m amp., chart speed 0.76 m/hr with a hydrogen flow of 70 ml/min.

Linseed oil methyl ester was used as an standard, for internal standardization. Pure samples of lipid standards were purchased from Sigma Chemical Company, U.S.A.

(vii) Infrared: IR spectra were determined with Perkin-Elmer Model 621 spectrophotometer as liquid film or as 1% solution in carbon tetrachloride or carbon disulphide.

(viii) Ultraviolet: A Beckman DK-2A instrument was used to determine UV spectra in methanol.

A new epoxy acid of Mucuna prurita Seed oil

Epoxy fatty acids are well distributed in seed oils of various plant families, out of which four viz., Compositae, Dipsacaceae, Euphorbiaceae and Malvaceae, may be considered of major importance, since they incorporate large number of species with seed oils more or less rich in epoxy acids[135]. The family Leguminosae has been reported to have two members, Calliandra eriophylla and Cercidium floridum, containing appreciable amounts of epoxy acids in their seed oils[135]. One more member, Glycine hispida (Soybean) has been shown to contain about 0.3% of monoepoxy glyceride from freshly harvested USA soybeans, whereas oil from stored beans contain more of them (generally 1-2%)[32]. A fourth member, Mucuna pruriens has been reported from our laboratory to contain 1.3% epoxide[59]. Previous work[136] on M. pruriens seed oil revealed the presence of only usual fatty acids (stearic, palmitic, myristic, arachidic, oleic, linoleic and linolenic). M. prurita (Legumnosae, Hindi Jangli Kawanch) seed oil has now been examined for its epoxide content. M. prurita is a wild variety of species M. pruriens which is cultivated in some parts of the country for its brown valvety legumes, which are cooked and eaten as a vegetable. Oil from the seed of M. prurita contains previously unidentified cis-12, 13-epoxyoctadec-trans-9-enoic (1%) and the more common vernolic

(cis-12,13-epoxyoctadec-cis-9-enoic) (4 %) acid. Gunstone's procedure of direct acetolysis of oil containing epoxy acids in minor amounts was adopted to characterize the epoxy acids in the oil. The techniques used in isolation and identification of the acids included elemental analysis, thin-layer and column chromatography, IR, UV, NMR and chemical reactions (viz., *reduction and oxidative cleavage) coupled with GLC.*

The M. prurita seeds contained 4.2% oil (dry basis). The UV spectrum showed no conjugation in the oil and its methyl ester. The IR spectrum of the oil showed a very weak band at 940 cm^{-1}, attributed to the epoxy group[137]. Picric acid TLC[84] also revealed the presence of epoxy function. The epoxy content in the oil was found to be 5% (based on HBr-titration and partitioning). The possibility for the occurrence of oxygenated acids other than epoxy in the oil was ruled out on the basis of IR and TLC.

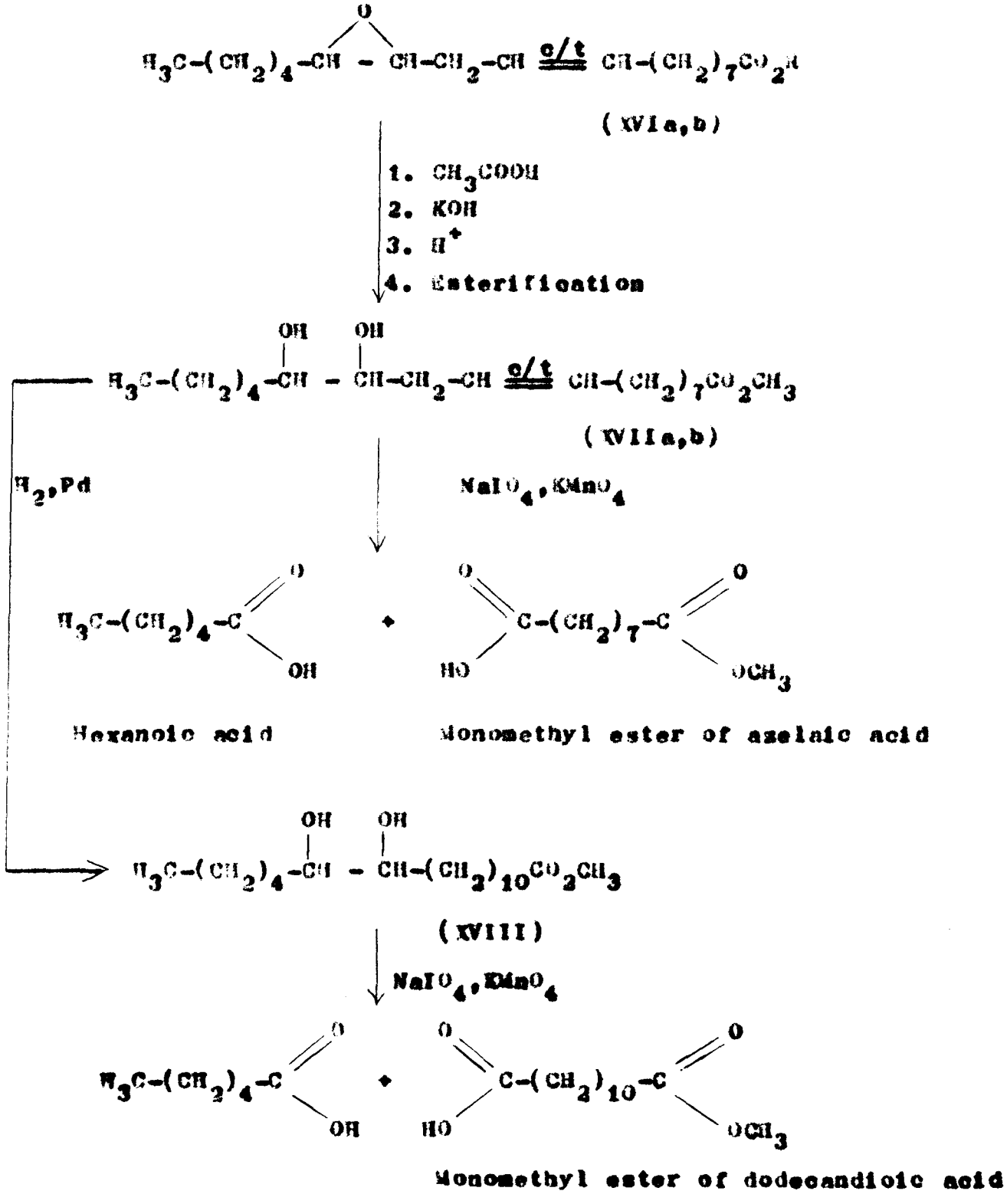
Characterization of epoxy acids

The epoxy acids of the oil were characterized as their corresponding dihydroxy acids formed by the direct acetolysis of oil followed by saponification[18]. The mixed dihydroxy esters on ordinary TLC migrated as a single spot whereas on Ag^{+}/TLC gave two spots differing in their R_f values. Separation of

these mixed dihydroxy esters by argentation column chromatography gave two fractions (major Fraction 1, 80%; minor Fraction 2, 20% by wt. of the total hydroxy esters).

Characterization of Fraction 1

The structure of the major dihydroxy ester (Fraction 1) was established by elemental analysis, catalytical hydrogenation, oxidative cleavage, and comparison of its TLC, IR and NMR characteristics with that of an authentic sample of methyl threo-12,13-dihydroxyoctadec-cis-9-enoate prepared from V. anthelmintica seed oil by the same treatment. Anal. Calcd for $C_{19}H_{36}O_4$: C, 69.51; H, 10.97. Found: C, 69.84; H, 10.91. It absorbed one mole of hydrogen (one double bond). A co-TLC on ordinary, silver nitrate and boric acid-impregnated plates with an authentic sample showed the identity of the two dihydroxy esters. The IR and NMR spectra were superimposable with that of authentic sample. Permanganate-periodate cleavage[13d] of this fraction as such and after hydrogenation also confirmed the proposed structure (Scheme 1). Therefore, the structure of Fraction 1 is established as methyl threo-12,13-dihydroxy-octadec-cis-9-enoate (XVIIa).



R=diacyl glyceryl moiety; XVIa and XVIIa = cis double bond
XVIb and XVIIb = trans double bond

Scheme 1

Characterization of Fraction 2

Anal. Calcd for $C_{19}H_{36}O_4$: C, 69.31; H, 10.97. Found: C, 69.45; H, 11.0. Absorbed one mole of hydrogen (one double bond). On Ag^+/TLC Fraction 2 (minor) migrated ahead of Fraction 1 (major). Comparison of this fraction with that of standard diol (from V. anthelmintica) by TLC on boric acid-impregnated Silica Gel G demonstrated that this had also threo configuration. The IR spectrum showed the bands at 3450 (due to -OH), 1740 (due to ester $-\overset{O}{\overset{\|}{C}}-$ group) and 970 (due to trans double bond) cm^{-1}. The ^1H NMR gave signals at τ 4.54 (3H, olefinic protons), 6.34 (s, 3H, ester methyl protons), 6.65 [4H, 2H (-CH-O) + 2H (-OH)], 7.76 (2H, α to the carbonyl function), 8.0 (protons α to the double bond), 8.7 (br s, shielded methylene protons), 9.12 (rough t, 3H, terminal methyl protons). On D_2O shake the signal at 6.67 was reduced and integrated for two protons only (2H, -CH-O) indicating that the hydroxyl protons signal was merged with the signal of -CH-O. When subjected to splitting by permanganate-periodate[138] as such and after hydrogenation, the cleavage products were the same as in the case of fraction 1 (Scheme 1). This indicated that the position of the hydroxyls and the double bond is the same in both the fractions. The presence of a distinct band near 970 cm^{-1} in the IR spectrum proves that the double bond is trans in this fraction. The most significant parameter in ^1H NMR for the determination of the configuration

of a double bond is the coupling constant between the alkenyl protons. But in this case, as for most isolated double bonds, it is not possible to measure coupling constant, but a small chemical shift difference in alkenyl protons of Fraction 2 (τ 4.54) and authentic sample (τ 4.6) giving different and distinct absorption is a conclusive evidence for the presence of trans and cis double bonds in Fraction 2 and authentic sample respectively[139]. On the basis of elemental analysis, reduction, oxidative cleavage, direct, argentation and boric acid TLC, IR and NMR data Fraction 2 was found to be methyl threo-12,13-dihydroxyoctadec-trans-9-enoate (XVIIb).

As no dihydroxy acid was obtained from the whole oil by solvent partition procedure when the acetolysis step is omitted, the dihydroxy acids are not present in the crude oil but must be formed by acetolysis of the epoxy acids. The presence of weak IR band at 970 cm^{-1} in the oxygenated oil concentrate showed that the acid with trans unsaturation is not an artifact formed during the chemical treatment of the oil (acetolysis followed by saponification) but is originally present in the oil.

The dihydroxy esters (Fractions 1 and 2) derived from the epoxy acids of M. prurita oil were identified as methyl threo-12,13-dihydroxyoctadec-cis-9-enoate (XVIIa) and methyl threo-12,13-dihydroxyoctadec-trans-9-enoate (XVIIb). These results demonstrate conclusively that the original epoxy acids are

cis-12,13-epoxyoctadec-*cis*-9-enoic (XVIa, Vernolic) and *cis*-12,13-epoxyoctadec-*trans*-9-enoic (XVIb) acids.

EXPERIMENTAL PROCEDURES

Picric acid TLC[84]

Picric acid TLC was carried out using Silica Gel G plate (2.5 x 8.5 cm). The developing system was petroleum ether (bp 40-60°)-ether-acetic acid (75:25:1, V/V/V). The plate, after developing was sprayed thoroughly with M picric acid in 95% ethanol and immediately placed in a tank saturated with the vapours of ether-ethyl alcohol (95%)(90:20, V/V). 30 min later the plate was removed and exposed to ammonia fumes for 1-2 min. The orange spot on a yellow background of the TLC plate indicated the presence of epoxy group.

Hydrogen bromide titration[140]

The quantitation of total epoxy acid content was carried out by titration of weighed amount of oil with 0.1N hydrogen bromide solution using crystal violet as indicator at 3°C to a bluish green end point that persists for 30 sec by the procedure of Harris *et al.*[140] The amount of epoxy acid was calculated as epoxyoleic. The percentage of oxirane oxygen

and epoxy oleic was calculated by the equations:

$$\% \text{ of Oxirane Oxygen} = \frac{V \times N \times 1.60}{\text{wt. of sample}}$$

where N = Normality of HBr; V=Volume of HBr consumed in titration.

0.2% Oxirane oxygen = 3.9% Epoxy oleic.

Thin-layer chromatography

TLC analyses of the oil as well as the esters were done on Silica Gel G plates (0.25 or 1.0 mm thick) developed with ether-hexane-acetic acid (20:80:1, V/V/V). Dihydroxy acid methyl esters were also analyzed on Silica Gel G impregnated with boric acid[93], developed with ether-hexane-acetic acid (40:60:1, V/V/V) and Silica Gel G impregnated with $AgNO_3$[94] and developed with ether-hexane (40:60, V/V). The spots on all analytical TLC plates were visualized by charring them at 120° after spraying with a saturated solution of 50% chromic acid.

Column chromatography

Mixed dihydroxy esters were fractionated by argentation column chromatography. Silver nitrate-impregnated Silica gel was made into a thick slurry with hexane and poured into a chromatographic column. Elution was carried out with increasing percentages of ether in hexane.

Gas-liquid chromatography

GLC analyses of methyl esters were carried out as described by Miwa[134]. A Perkin-Elmer Model 154, equipped with thermal conductivity detector, using stainless steel packed column (2m x 3 mm) coated with diethyleneglycol succinate (DEGS, 15% on chromosorb W, 45-60 mesh) was employed. The separations were carried out at temperatures of 100 and 200°, chart speed 0.76 m/hr with a hydrogen flow of 70 ml/min.

Sample preparation

Oil was extracted from the ground seeds with petroleum ether (bp 40-60°) and the methyl esters prepared by NaOMe transesterification[141]. A portion of the oil was acetolysed with five volumes of glacial acetic acid for 7 hr as described by Gunstone[25]. The mixture was then diluted with water and extracted with ether . The combined ether extracts were dried over anhydrous sodium sulphate and evaporated in vacuo to yield a viscous oil. Saponification of the recovered oil was effected with 1N-KOH/EtOH. The unsaponifiable material was removed and then acidified with HCl to liberate the fatty acids. Separation of these mixed fatty acids into oxygenated and non-oxygenated fractions was accomplished by partition between 80% methanol and petroleum ether (bp 40-60°). The

methanol extract was re-extracted with petroleum ether (4 x 50 ml) to remove any trace of non-oxygenated acids. Direct TLC revealed a clear spot on the base line, thus showing the presence of oxygenated acid in the fraction isolated from methanolic phase. The crude dihydroxy acids thus isolated from the methanolic phase and the concentrate obtained from petroleum ether extracts were converted to their methyl esters by sodium methoxide catalyzed methanolysis[141].

Hydrogenation and Oxidative splitting of the dihydroxy acids

The hydroxy esters were hydrogenated in methanol with Adams catalyst. In order to locate the position of the original epoxy group and the double bond the dihydroxy esters were cleaved before and after hydrogenation by the permanganate periodate method of von Rudloff[138] and the products were subsequently chromatographed on GLC columns after esterification.

Spectrometry

IR spectra were determined with Perkin-Elmer Model 621 spectrometer. NMR spectra were run in CCl_4 at 60 MHz with TMS as internal standard, chemical shifts are expressed in τ . A Beckman DK-2A instrument was used to determine UV spectra.

The analytical values of seeds and oil, determined according to the procedures recommended by the AOCS method[133]

are summarised in Table III. The nonoxygenated esters when analyzed by GLC, had the composition shown in Table IV.

TABLE III

Analytical data on *M. prurita* seeds and oil

Oil %	4.2
Moisture %	5.6
Protein content N x 6.25%	30.0
Refractive index, n_D^{40}	1.4840
Iodine value (Wijs)	98.0
Saponification value	194.0
Oxirane oxygen, %	0.25
HBr equiv. (% epoxyoleic)	4.87
Unsaponifiable content %	2.8
Ultraviolet (UV)	Usual
Infrared (IR)	840 cm^{-1}

TABLE IV

Fatty acid composition of *M. prurita* oil

Component	%
16:0	21.0
18:0	2.7
18:1	16.8
18:2	46.1
18:3	8.4
Vernolic	4.0*
cis-12,13-epoxyoctadec-*trans*-9-enoic	1.0*

* Calculated with the help of HBr titration, partitioning, and column separation.

Cyanolipids of Aesculus indica seed oil

Cyanolipids are not glycerides but instead are derivatives of five-carbon mono- or dihydroxynitrile moiety esterified with long-chain fatty acids. Sporadic reports have appeared recently regarding the co-occurrence of cyanogenic nonglycerol esters with seed oil triglycerides. Way back to 1920, probably cyanolipids were first observed in Schleichera trijuga (Sapindaceae) seed oil by Rosenthaler[142] and Sen-Gupta[143]. But the exact location of the cyanogenic compound in the oil or its exact nature was not reported. The compound has been suspected to be in the form of a cyanogenic glucoside or an acid amide[144]. Later, Kundu and Bandyopadhyay[90] reinvestigated the same seed oil to ascertain the location and nature of the cyanogenic compound by applying chemical methods, chromatography and infrared spectroscopy. Observations indicated the cyanogenic compounds to be a part of glyceride molecules in which one of the hydroxyl groups of the latter is bonded to the cyanogenic compound through an ether linkage. Chromatographic behaviour of the isolated cyanogenic compounds further indicated that at least two glyceride molecules are involved. These glycerides are predominantly esterified with saturated fatty acids. At the same time Kasbekar and Bringi[145] working on the same seed oil found with the help of TLC that the oil is composed of approximately 37% of glyceride, the rest being nonglycerol esters of fatty acids. Recent

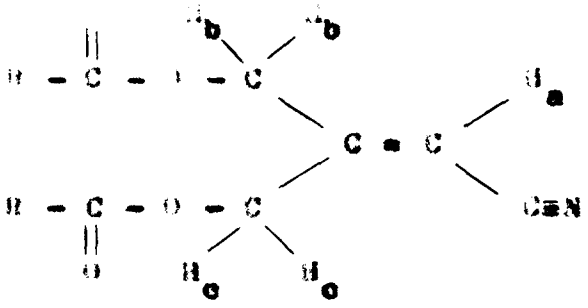
studies[68-76] have shown that the cyanogenic material is a nonglycerol ester composed of one or two ordinary fatty acid moieties (predominantly C_{20}) esterified with an unsaturated isoprenoid hydroxy- or dihydroxynitrile. Four types of cyanolipids (XII to XV) present individually or in pairs have been identified in the seed lipids of the Boraginaceae and Sapindaceae species. More recently the presence of cyanolipids has been shown from our laboratory in Cardiospermum canescens (Sapindaceae)[77], Dodonea viscosa (Sapindaceae)[79] and two species of Heliotropium (Boraginaceae)[78].

The present work deals with the isolation, identification and composition of one such cyanolipid, namely, the fatty acid diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol (XIII)(20 %, w/w) in the seed oil of Aesculus indica colebr (a Indian horsechestnut). Although the nitrogen-containing lipid fraction (NCLF) is nonhomogeneous, the constituents differ only in the attached fatty acids; hence, this mixture was treated as a single entity throughout this investigation. Previously, according to Bentham and Hooker (1862-1883) this plant was classified with family Sapindaceae but now Engler and Prantl (1887-1915) and Hutchinson (1926-1959) put this plant with the family Hippocastanaceae. The fatty composition of the cyanolipid component was also compared with that of triglycerides.

Aesculus indica seed kernels contained 4% of oil. Preliminary TLC analysis of the seed oil using soybean oil as standard revealed the presence of usual components. The oil gave the positive picrate and prussian blue colour tests. Elemental analysis showed the presence of 2.2% of nitrogen. On Silica Gel G TLC, the oil gave two spots (triglycerides, Rf, 0.77 and cyanolipid, Rf, 0.52) using hexane-ether-acetic acid (85:15:1, V/V/V) as solvent system but only a single spot with benzene. The oil was resolved into a triglyceride fraction (80%, W/W) and a cyanolipid fraction (20%, W/W) by preparative-TLC using ether-hexane (1:3) as developing solvent.

Treatment of NCLF with dilute base generated HCN as shown by positive picrate[149] and Prussian blue[150] tests. The structure of the cyanolipid derived from A. indica seed oil was established by a comparison of its TLC, IR and NMR data with those reported for the corresponding cyanolipid present in the seed oils of Sapindaceae. On running a co-TLC of the isolated NCLF with the cyanolipid of Cardiospermum halicacabum seed oil, it was observed that the NCLF exactly corresponded to the minor cyanolipid constituent of the standard oil. This TLC behaviour suggested that the cyanolipid is likely to be a diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol. The IR (1% solution in CS_2) analysis of the NCLF revealed a nitrile band of medium intensity at 2220 (C≡N) cm^{-1} and the IR spectrum was superimposable on the spectrum of corresponding cyanolipid isolated from C. halicacabum seed oil.

The NMR spectrum of the cyanogenetic lipid material from oil was particularly informative with regards to the structural features of the nitrile-containing moiety. The NMR spectrum of the cyanolipid revealed proton counts, chemical shifts and multiplicities identical with those displayed by reference sample of fatty acid diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol isolated from C. hallicacabum seed oil. The NMR spectrum exhibited signals characteristic for long-chain lipid groups, τ 9.12 (rough t, 3H, terminal methyl), 8.75 (br s, shielded methylene), 8.05-7.97 (m, protons α to the double bond), 7.67 (t, protons α to the carbonyl function) and 4.7 (rough t, olefinic protons). The two sets of methylene protons H_b and H_c (XIII) which are adjacent to the oxygen atoms of the dihydroxynitrile moiety, gave the signals at τ 5.93 and 5.93. This difference in shielding is caused by the stereochemistry of the methylene groups; one of them is cis to the nitrile grouping and the other is trans. As a result of the stereochemical difference between the two methylene groups, the protons of one group couple more strongly with the vinyl proton (τ 4.45) than to protons of the other methylene group. The cyanohydrin proton (H_a) appeared as a slightly broadened singlet at τ 4.45. The comparative TLC and IR characteristics coupled with NMR data established that the cyanolipid present in the oil is a fatty acid diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol identical to the minor NCLF of C. hallicacabum.



(XIII)

The lipid groups of the triglycerides as well as cyanolipid constituent of the oil (Table V) were identified by converting them to their methyl esters by transesterification or acid-catalyzed methylation and comparing the methyl esters by GLC with authentic standards. On comparing it was found that a higher proportion of C_{20} acids occur in the cyanolipids than in the triglycerides.

TABLE V

Fraction	Fatty acid Composition							
	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0
Triglyceride	13.9	2.3	41.0	23.3	2.6	7.0	7.8	1.2
Cyanolipid	8.4	1.4	29.6	16.6	trace	18.0	24.0	5.0

Because of its basically isoprenoid structure, the dihydroxynitrile moiety of NCLF has many biogenetic possibilities. It may be related, perhaps somewhat remotely, to biological compounds such as cordycepose[146] or mevaldic acid[147]. However, rather extensive studies made on the biosynthesis of other cyanogenetic materials[81-93] indicate that most of them are derived from amino acids or their precursors.

EXPERIMENTAL PROCEDURES

Oil recovery and methyl ester formation

Oil was recovered from finely ground seed by a 16 hr extraction with petroleum ether (bp 40-60°) in a Soxhlet apparatus. The methyl esters were prepared using 1% NaOMe in MeOH or acid-catalyzed methylation.

Apparatus

Infrared (IR) spectra were determined with Perkin-Elmer Model 621 spectrophotometers on 1% solutions in CS_2, $CHCl_3$ or CCl_4. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian A-60D spectrometer; the solvent used was CCl_4. All reported chemical shifts are measured from internal tetramethylsilane (TMS)= τ 10.0. A Beckman DK-2A instrument was used to determine UV spectra.

GLC analyses of methyl esters were performed essentially as described by Miwa and coworkers[134] by using stainless steel packed column (2 m x 3 mm) coated with diethylene glycol succinate (DEGS, 15% on chromosorb, W, 45-60 mesh). A Perkin-Elmer Model 154 vapour Fractometer was employed in these analyses and the separations were carried out isothermally at 200°C, with a hydrogen flow rate of 70 ml/min.

Thin-layer chromatography

Thin-layer chromatography (TLC) was done on 0.25 mm layers of Silica Gel G developed with solvent system of benzene or hexane-ether-acetic acid (85:15:1, V/V/V). Spots were detected by charring the plates after they had been sprayed with a saturated solution of CrO_3 in 50% aqueous H_2SO_4. The oil was resolved into a triglyceride fraction and a cyanolipid fraction by preparative TLC. For preparative TLC separation plates 20 x 40 cm with Silica Gel G layers, 1 mm thick were used. The solvent was ether-hexane (1:2, V/V). The spots were detected by spraying with an alcoholic solution of 2',7'-dichlorofluorescein and viewing them under ultraviolet (UV) light. Desired constituents were recovered from the silica by standard procedures and the purity of the fractions was checked by analytical TLC.

Formation and detection of HCN

Two tests were used to detect HCN derived from seed oil and NCLF. One of these, the picrate test, depends on the reaction of HCN with alkaline picrate solution to produce isopurpuric acid.[145] About 75–100 mg of lipid material was placed in a test tube with 1 ml of dilute NaOH or H_2SO_4. A strip of filter paper dipped in an alkaline solution of sodium picrate (0.5%) was partially dried and was then suspended over the mixture in the stoppered test tube. Test tube and contents were warmed at 35–50° for 0.5–1 hr. A positive test involves a colour change of the filter paper from yellow to brick red[149].

The second test involved formation of Prussian blue[150]. Material to be tested was placed in a 50 ml Erlenmeyer flask with 2 ml of methanol and either 1 ml of 10% NaOH or 1 ml of 6N H_2SO_4. If NaOH was used, the mixture was heated a few minutes in a hot water bath and acidified with H_2SO_4. A filter paper moistened with NaOH solution was placed over the mouth of the flask and the flask was warmed 5–10 min. After the filter paper was removed, it was treated with three drops of 3% ferrous sulfate solution and, when nearly dry, with 10% HCl. An intense Prussian blue colour indicated a positive test for cyanide.

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PART - II

REACTIONS OF NITROSYL CHLORIDE WITH LONG-CHAIN FATTY ACIDS AND THEIR DERIVATIVES

A. THEORETICAL

We are gradually increasing our awareness and understanding of the wide variety of reactions which fatty acids undergo, especially those which are unsaturated. At a simple level one might expect that any reaction observed on a short-chain acid or alkene should be applicable to longer-chain substrates whether natural or synthetic.

Reactions of fatty acids in general are associated with (i) the carboxyl group and (ii) the hydrocarbon chain. During the first quarter of present century very little was known about the mechanism and stereochemistry of reactions of double bond. With the growing understanding of the mechanism of organic reactions, the controversial problems of organic chemistry were gradually solved.

Among the reactions involving the hydrocarbon chain of fatty acids those of oxidation, halogenation and hydrogenation are of fundamental importance in fat chemistry. The large variety of products resulting from these reactions of an unusual fatty acid has been the main drawback in its systematic study. The opportunities which exist require that extreme care should be taken in their preparation, isolation and in selecting the criteria of purity.

A survey of the literature reveals that the results obtained by different groups of workers at different times have led to the interpretations which are conflicting, as far as the mechanism and stereochemistry are concerned.

It is now realized that organic chemistry is, to a large extent, the study of reactions of functional groups with important contribution of polar, steric, conformational and neighbouring groups effects. During the last two decades, in particular, new and interesting reactions of fatty acids have been described that provide new route to the synthesis of a variety of fatty acid derivatives. The growing demand of fatty chemicals as intermediate raw materials has diverted the attention of lipid chemists from the analytical aspect of fats to the chemistry of unusual fatty acids.

Most recent developments in the chemistry of fatty acids begin from 1960 to date. This period is characterized by a series of investigations on the non-classical reactions of fatty acids. These non-classical reactions include oxymercuration-demercuration, rearrangement of 1,2-epoxide, cyclodehydration (1,4-epoxide) of hydroxy olefinic acids, allylic halogenation and oxidation of olefinic acids, cyclopropanation and reactions leading to the synthesis of nitrogen and sulphur analogues of the oxygenated acids. The growth of organic nitrogen chemistry has been rapid, and it not only shows no signs of abatement,

but the literature has been proliferating at an increasing rate. Aside from the intellectual challenge involved in preparing novel organic nitrogen compounds of the most amazing complexity and structural ramifications, organic chemists should be deeply concerned with nitrogen compounds because of their widespread use and intrinsic importance[1].

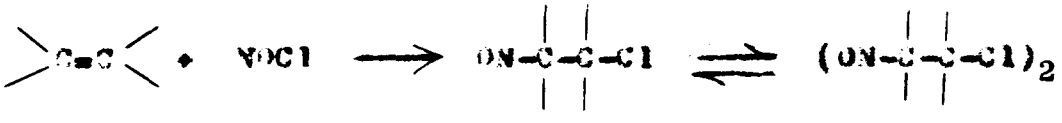
Nitrosyl chloride (NOCl) addition represents one of the simplest ways of elaborating a carbon-nitrogen bond directly from unsaturated compounds. The reaction of nitrosyl chloride with olefins has been known for almost 100 years, and an extensive wealth of literature has accumulated on this subject. Nitrosyl chloride reacts with most of the elements and with an extremely wide range of compounds. Comprehensive literature reviews[2-4] have summarized the present state of knowledge and it is necessary here to highlight some of the salient features of the NOCl reaction upon organic compounds including fatty acids.

Reactions of nitrosyl chloride with carbon-to-carbon multiple bonds

The reactions of this classification involve principally the addition of nitrosyl chloride to double bonds, i.e. nitrosochlorination. During the present century less emphasis has been placed on reactions with terpenes and more study has been devoted to the applications of nitrosyl chloride in the treatment of complex natural products and to its use in the synthesis of detergents and other materials.

Olefins add on nitrosyl chloride where the chlorine atom being the negative end of the dipole in NOCl will add on to the carbon atom joined to the least number of hydrogen atoms (Markownikoff's rule).

The reaction of olefins with NOCl to give nitroso chloride which dimerize if unhindered (eq.1) has been known since long[5], and has played an important roles in early studies of terpenes. The use of an alkylnitrite and hydrochloric acid, generating the nitrosyl chloride <u>in situ</u>, provides a convenient, alternate technique for carrying out these additions. Nitroso compounds are usually blue or green liquids which dimerize to white crystalline solids often in equilibrium with the monomeric form. These bimolecular solids regenerate the monomer when fused or when dissolved in solution. Chilton <u>et al.</u> (1953) and Gowenlock <u>et al.</u> (1955)[6] have found that these dimers have <u>cis</u> or <u>trans</u> configuration. Aliphatic <u>trans</u> dimers exhibit infrared absorption in the region 1290–1176 cm^{-1} whereas <u>cis</u> dimer have absorption in the region 1420–1330 and 1344–1323 cm^{-1}[7]. Monomeric C-nitroso compounds absorb in the region of 1493–1520 cm^{-1}[7].



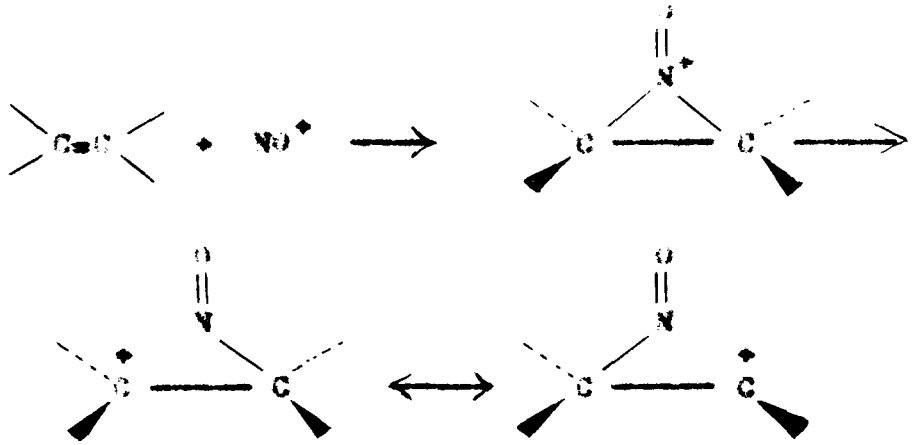
(1)

<u>cis</u> <u>trans</u>

Isomerization to oxidic structures yielding chloroximes is feasible where the labile hydrogen on the carbon of nitroso group attachment is available (eq. 2). The oximes are more stable since bonds between heteroatoms are always weak, and the oxime has only one such bond while nitroso has two.

(2)

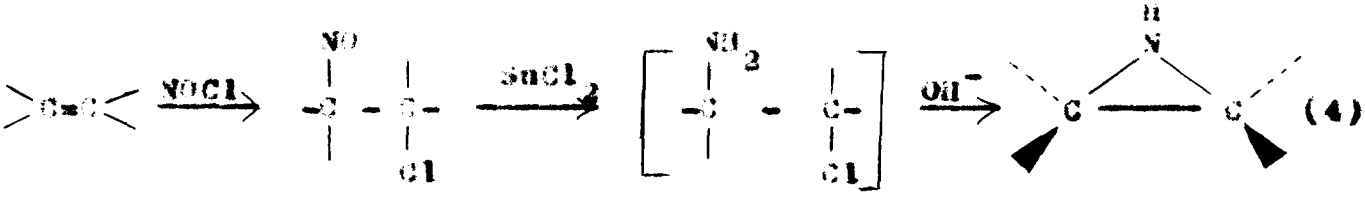
Mechanism involving NO^+ and Cl^- has been generally assumed for nitrosyl chloride addition to double bond[3,9]. For example, Kaplan, Kwart, and Schleyer[3] have suggested that nitrosyl chloride ionize to give the nitrosonium ion (NO^+) which adds to an olefin to give a highly stabilized onium ion intermediate (eq. 3), which should open to give a trans nitrosochloride.



(3)

A novel preparation of aziridines from tetrasubstituted olefins[10], consisting of nitrosyl chloride addition followed by

stannous chloride reduction to give a chloroamine and base cyclization (eq.4) is consistent with this proposal.



(4)

Since a knowledge of the stereochemistry of nitrosyl halide additions should increase the synthetic utility of these reactions as well as help in elucidating their mechanisms, Heinzelmann et al.[11] studied this problem explicitly for a variety of olefins. They found that the steric course of nitrosyl halide addition to olefins depends on the olefin structure. Thus, Δ^9-octalin gives a trans adduct, in accord with the generally assumed ionic reaction mechanism, and it is probably that most other unstrained olefins behave similarly[12]. On the other hand, the addition of nitrosyl chloride and nitrosyl bromide to norbornene, anti-7-methoxynorbornene and norbornadiene follows a cis stereochemical course and is unaccompanied by molecular rearrangement[13-15] suggesting that if these reactions are ionic ones, very little electron demand is made on these olefins in the transition state. There is a close similarity between the pattern of reactivity uncovered in this work and that shown in the oxymercurization reaction. Unstrained olefins undergo trans addition via an electrophilic mechanisms, but certain strained alkenes such as norbornene have been shown to

give a cis-oxygenation products[16]. Meinwald et al.[11] postulated a single mechanism to accommodate both cases. As a first step, the olefin would react with the nitrosyl halide to give an onium ion, as discussed above, with the cyclic contributing structure being the most important. For an intermediate in which trans displacement of one of the C-N bonds is sterically acceptable, the cyclic intermediate is opened by attack of halide ion to give, a trans product. For a more constrained substrate, in which such a trans displacement would require a difficult twisting about a C-C bond in a relatively inflexible system, it may be postulated that attack of halide ion from a cis position is more favourable, and a cis adduct results.

A decision between these possibilities does not seem possible on the basis of the data now available. It is hoped, however, that the demonstration of a relationship between the structure of an olefin and the stereochemistry of its derived nitrosyl halide adduct may prove useful.

In contrast to the nitrosyl chloride addition reactions which take place with olefinic groups at low temperature, chlorination or oxidation effects are obtained at elevated temperatures and in some cases even at room temperature. The reported formation of nitro derivatives from nitrosyl chloride and some chlorinated olefins[17] apparently involves oxidation of the initially formed nitroso compound.

addition of nitrosyl chloride to higher molecular weight olefins or derivatives and improvements in the procedures have formed the basis of a number of patents for the manufacture of surface-active agents. Generally the nitrosyl chloride addition products of the high-molecular-weight olefins are found to be liquids.

Although addition of nitrosyl chloride to terpenes and the simple olefins has been widely studied[3], addition to unsaturated fatty acid derivatives has received little attention. It was shown in 1894 by Tilden and Forster[18] that nitrosyl chloride adds to oleic and elaidic acids quite readily but their isolation of solid products seems improbable in light of the work reported by Miller et al.[19] A patent[20] has disclosed the preparation of surfactants from nitrosyl chloride adducts of oleic acid and its esters. Of particular interest is a paper by Kaufmann and Mower[21] reporting an analytical method for unsaturated fatty materials based on addition of nitrosyl chloride in a manner analogous to that used with iodine monochloride in the standard iodine value determination[22]. However, only the disappearance of NOCl was measured, and no attempts were made to isolate products. They state that their studies would be directed toward preparative work based on nitrosyl chloride adducts of unsaturated fatty materials. Miller et al.[19] later on, reported the successful addition of nitrosyl chloride to methyl oleate on a preparative scale (at 2° using methylene chloride as solvent)

and some reactions of the product. The product, methyl 9(10)-chloro-10(9)-nitrosostearate, is formed according to equation (5).

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_3 + NOCl \longrightarrow CH_3(CH_2)_7\underset{\underset{(Cl)}{|}}{\underset{NO}{|}}{CH} - \underset{\underset{(NO)}{|}}{\underset{Cl}{|}}{CH}(CH_2)_7CO_2CH_3 \qquad (5)$$

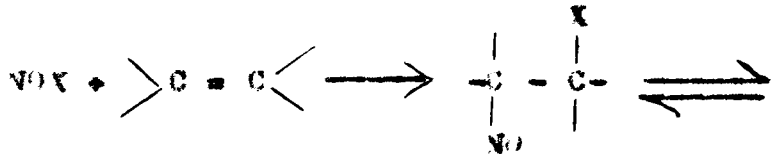
The dimeric product which is so often observed in the reactions of nitrosyl chloride with olefins is not formed in significant amounts under these conditions. The usually facile rearrangement of the secondary nitroso compound to the oxime (eq. 6) proceeds slowly on standing and is not easily accelerated.

$$\begin{array}{c} -CH-\\ |\\ Cl \end{array} \begin{array}{c} -CH-\\ |\\ NO \end{array} \longrightarrow \begin{array}{c} -CH-\\ |\\ Cl \end{array} \begin{array}{c} -C-\\ \|\\ NOH \end{array} \qquad (6)$$

The stereochemical course of the nitrosochlorination of methyl oleate has not been investigated, and the literature concerning the nitrosochlorination of other olefins does not provide a satisfactory guide. Undoubtedly, the nitrosochlorination of unsaturated fatty acids and other long-chain aliphatic olefins needs further investigation, since it is potentially useful in the synthesis of fatty nitrogen derivatives. A superficial examination of some of the reactions of methyl chloronitrosostearate has indicated that a variety of products can be prepared[19] and has shown the value of this type of adduct as an intermediate. Not yet demonstrated, but certainly

within the realm of reasonable possibility, is the conversion of fatty chloronitroso derivatives, to the valuable aziridines (eq.4) and nitroaziridines.[34]

The addition reaction of nitrosyl chloride with olefins, with subsequent hydrolysis of the adducts with levulinic acid made 0.1N in hydrochloric acid has been shown to be a convenient general method for converting olefins to the corresponding chloro ketones[23] (eq.7). Hydrolysis presumably proceeds via oxime

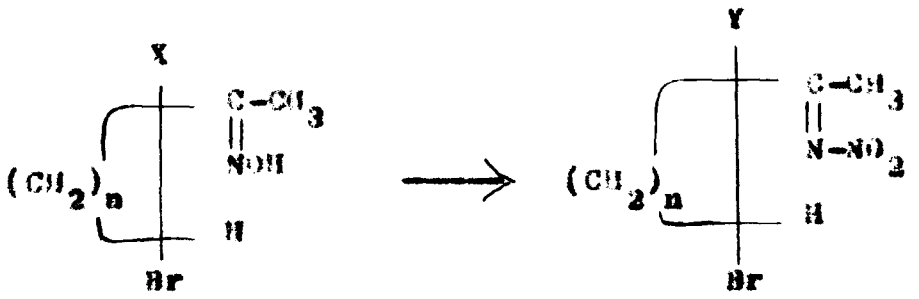


(7)

tautomer of the monomeric nitroso compound. There is all possibility for converting olefinic fatty acids to the corresponding chloro ketones by the application of this reaction.

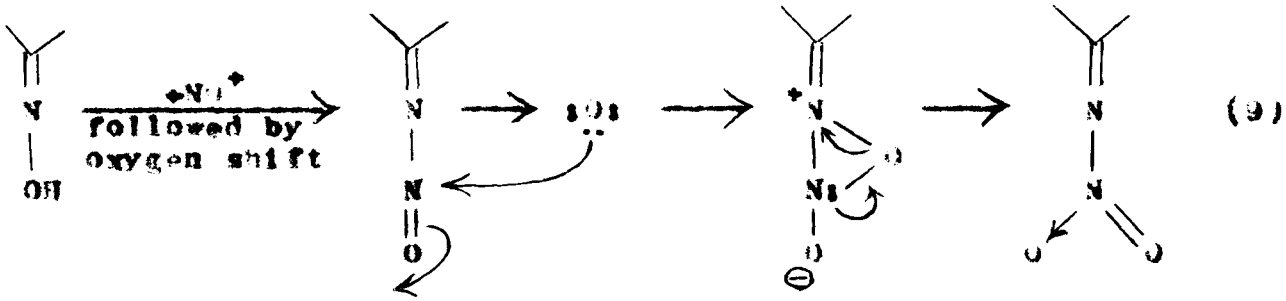
Normal addition of nitrosyl chloride to an olefin gives a chloronitroso product (monomer or dimer) or an α-chlorooxime. Other products have been called anomalous[3,4]. The normal (primary) products may be oxidized to secondary products. Shine et al.[24] have found a quite different result when compounds of type I are treated with NOCl. Two reactions occur. The oximino group is oxidized to a nitramine (II)(eq. 8), a result

that has been accomplished by nitrous acid oxidation[25,26] and by nitrosyl flouride[27] but not by NOCl. The oxidizing action of NOCl has been established.



(I) (II) (8)

The mechanism of nitrimine formation (new N-N bond formation at the oximino nitrogen by a electrophilic NO^+ group, followed by an oxygen shift) suggested by Freeman,[25,26] and supported by Boswell[37] seems adequate to account for these results (eq. 9).



(9)

The sharp OH absorption at 3600 cm^{-1} characteristic of oximes[38] in dilute CCl_4 solution disappears as the reaction occurs. Compound II show strong bands at 1590 and 1320 cm^{-1} (NO_2) and medium bands at 1640 cm^{-1} (C=N), characteristic of nitrimines[39]. In the following year, 1971, Shiue et al.[30] reported additions to two ethylidenecycloalkanes and concluded

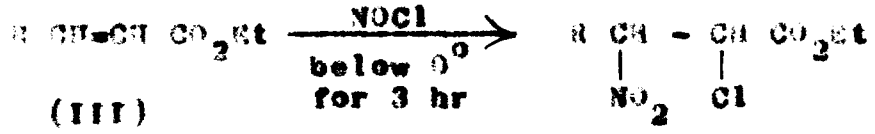
that the chloronitroso addition is the only primary reaction. After that three pathways may be followed: (1) dimerization of the nitroso group (long known), (2) oxidation of the nitroso group to a nitro group, and (3) isomerization to an oxime, followed by oxidation to nitrimine.

Three pathways compete. The second pathway appeared to be the only one in steroid example[31,32] where dimerization may be inhibited or very slow. Oxidation of an oxime to a nitrimine has been accomplished recently by nitrosyl chloride[24]. Isomerization of chloronitroso compound to the oxime is catalyzed by hydrogen chloride and goes very rapidly in polar solvents[30] so that dimerization and oxidation to a nitro group may not compete successfully in such solvents. The results reported by Shiue et al.[30] bear out Oglobins suggestion that stable dimer precipitation diminishes opportunity for oxidation to a nitro compound. Their work also suggests that rapid isomerization to oxime lowers nitro formation and increases nitrimine formation.

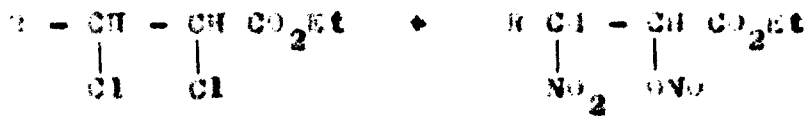
In the mass spectrometer, only one decomposition pattern is obtained from monomer dimer and oximino form. So the dimer must dissociate and/or isomerize in the ion chamber. The maximum m/e observed is that of nitrosyl chloride monomer[30].

In 1972 Shin et al.[33] carried out reaction of α,β-unsaturated carboxylic ester (III) with nitrosyl chloride and observed that the chief products are IV and V as shown in

reaction (10). The reaction was carried by adding nitrosyl chloride in a stream to a solution of compound III in dry benzene cooled below 0° , with an ice-salt bath. After being stirred for 3 hr at 0° , this reaction mixture was allowed to attain room temperature and to stand for 5 days.



(III) (IV) (10)



(V) (VI)

Recently the use of NOCl reaction on double bond has been made for the synthesis of N-nitroaziridine[34]. The reduction and subsequent base cyclization of nitramine formed by the action of excess NOCl upon steroidal compound provides a route for the synthesis of N-nitroaziridine which were hitherto known as unstable compounds.

Reaction with hydroxyl groups

In reactions with hydroxyl compounds, nitrosyl chloride functions as the acid chloride of nitrous acid, generally leading to the formation of nitrites. This reaction is identical in effect with the nitrosation of amino, methylene, and similar

hydrogen-containing groups. In some cases, oxidation of the hydroxyl group takes place, together with chlorination in other parts of the molecule.

In the gas phase reaction of methanol and nitrosyl chloride an equilibrium is instantly established[35] even at 25° (eq. 11).

$$ROH + NOCl \rightleftharpoons R\,ONO + HCl \qquad (11)$$

In view of the equilibrium, it is readily understood why alkyl nitrite plus hydrochloric acid formed a convenient means of preparing nitrosyl chloride <u>in situ</u> for organic reactions in much of the early work. Using dry pyridine on an acid acceptor in liquid-phase reaction makes high yields of nitrites possible from alcohols such as amyl, n-octyl[36], d-3-nonanol[37] and tertiary alcohols such as 3-methyl-3-pentanol and 3-ethyl-3-hexanol[38]. Under conditions used successfully with the above alcohols, glycerol, ethylene glycol, chloretone, menthol, trimethylene chlorohydrin, and benzyl alcohol do not yield nitrites[38]. It is of note that d-2-octanol in reaction with nitrosyl chloride gives an 80% yield of the dextrorotatory nitrite[39].

Hydroxyl groups attached to aromatic nuclei do not readily form nitrites.

Although much careful work has been done on the reactions of fatty acids, the search for purity and homogeneity was severely impeded by the lack of methods for determining the approach to this ideal state. Recent advances in chromatographic methods of separation and spectroscopic methods of structure determination make it possible that all the products of a reaction can now be examined profitably in fatty acids.

The use of spectroscopic methods has contributed much to our recognition of a variety of novel fatty acids and their derivatives and our understanding of their molecular structure and reactions. Out of the four spectroscopic disciplines, the use of NMR and mass in the study of fatty acid identification and characterization of their derivatives have attracted considerable attention in recent years. Therefore it is appropriate here to give a brief account of the application of NMR and mass spectrometry in the chemistry of fatty acids.

Nuclear Magnetic Resonance (NMR) Spectroscopy

From the late 1950's onward, applications of NMR spectroscopy have developed continuously, and they occupy a paramount position as a research tool in organic chemistry. The development of NMR has been characterized by a series of stages which have made it an increasingly powerful research technique and each of these has found an application to lipids. When the NMR spectrum of a simple molecule is determined, its chemical structure can often be elucidated by first order interpretation of spectral data. The number of different types of protons in the molecule can be determined by integration of peak area and information about proton environment can be obtained from the chemical shift, multiplicity, and coupling constants of distinguishable peaks. In recent years various techniques have been developed to extend the application of NMR to compounds of complex structure. Among these are: (a) addition of D_2O to suppress the signals of -OH and $-NH_2$ protons, (b) determination of spectra in various solvents to obtain information from solvent effects, (c) application of decoupling (double resonance or double irradiation) to simplify complex signals and to identify related protons, (d) repeated scanning and averaging by computer to obtain definite spectra in very small samples, (e) the use of shift reagents in structural determination, and (f) ^{13}C NMR.

A number of reviews[43-47] on the NMR spectra of fatty acids have appeared in the literature. The first commercially available NMR instruments were mostly of the 60 MHz variety and were primarily for recording proton spectra. It was recognized that 60 MHz NMR spectra of fatty methyl esters, including those of PUFA, contained signals which corresponded to groups of protons in various environments along the hydrocarbon chain[44,45].

Introduction of 100 MHz instrumentation was the next major advance in NMR, and this was followed within a few years by 220 MHz spectrometers. Each of these refinements resulted in a considerable enhancement in the resolution obtainable with a corresponding simplification of spectra. A comparison of 100 MHz ^{1}H NMR (PMR) spectrum of PUFA with those of 60 MHz spectrum reveals some sharpening of the various signals, especially the τ7.75 triplet due to protons α to the carboxyl group, and an apparent triplet centered at τ 8.3 associated with the metaylene group which is β to the carboxyl group and also β to a double bond. In 220 MHz NMR spectra, resolution of proton signals is enhanced to such a point that each PUFA tends to give a distinctive spectrum[46,47]. It has been shown[47] that 220 MHz spectroscopy can be used to determine both the stereochemistry and position of double bonds, and the position of triple bonds, in the majority of fatty acids and esters.

Although PMR is used by lipid chemists, the technique is severely limited in scope and utility because in most long-chain compounds, the majority of chain methylene protons, for all practical purposes, are magnetically equivalent. High resolution NMR spectroscopy, a powerful tool in many fields of organic chemistry, has been used to advantage in the study of some unsaturated fatty acids but has found limited application in the analysis of fatty acid derivatives due to coincident chemical shift of methylene protons. These protons yield a broad signal of overlapping resonances which preclude their identification and counting as well as the determination of their coupling constant. Since majority of the chain methylene protons are magnetically indistinguishable, it is impossible to confirm spectrally the presence or absence of chain substituents or chain branching. Recently, some interpretive problems have been overcome by determining the spectra in the presence of chemical shift reagents (CSR) which expand the NMR spectra of lipid derivatives, thus providing considerably more structural information than it has hitherto been possible to obtain. The best CSR developed so far are rare earth complexes of europium[48,49] or praseodymium[50]. Typical CSR complexes combine Eu(III) or Pr(III) with the anionic ligands: 2,2,6,6-tetramethyl-3,5-heptanedione or 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione; abbreviated designations for these complexes are $Eu(thd)_3$, $Pr(thd)_3$, $Eu(fod)_3$, and $Pr(fod)_3$. CSR can markedly

expand the spectra of compounds containing functional groups with lone pairs of electrons, if the lone pair can co-ordinate with the rare earth metal. The spectra are expanded because the chemical environment of protons near the co-ordination site is different from the environment of distant protons in the molecule. The signals of protons near the co-ordination site are therefore displaced. This displacement is directly related to the distance between the protons in question and the complexed metal atom; the smaller the distance, the greater the shift. Complexes containing Eu and Pr complement each other since, relative to tetramethylsilane (TMS), the Eu complexes shift proton signals downfield from their original position whereas Pr complexes shift them upfield.

If a molecule contains a functional group having sufficient Lewis basicity it can form a complex with CSR. The bonding in CSR complexes is considered to be mainly, if not exclusively, dipolar[50] and it has been reported to decrease in strength as the Lewis basicity of the functional group decreases: amines > alcohols > ketones ≥ aldehydes ≥ ethers > esters > nitriles; halide, indoles, and double bonds are inactive[49]. CSR induce changes in the NMR chemical shift of proton signals because the magnetic environment of protons in a complexed molecule differs from the magnetic environment of protons in an uncomplexed molecule. CSR complexation can often provide additional spectral data for protons upto eight carbons away from a CSR-active

functional group. Sometimes, however, due to overlapping NMR signals, useful information can only be obtained for protons within five carbons of a $Eu(fod)_3$ co-ordination site[31].

CSR reagents can substantially increase the amount of structural information obtainable from NMR studies of saturated and unsaturated lipid derivatives. It is theoretically possible to obtain more information from CSR studies of unsaturated lipid derivatives by introducing additional CSR-active functional groups into these molecules through derivatisation of their double bonds. However, additional CSR co-ordination site complicate spectral interpretation, because they increase the number of signals that overlap. The two model compounds viz., methyl ricinoleate and methyl 12-hydroxystearate were investigated[32] to test the feasibility of attempting other CSR analyses of polyfunctional molecules of unknown structure. The individual proton signals have been observed and assigned for all the protons in methyl ricinoleate, except those on carbons 5,6, and 7. Information obtained for methyl 12-hydroxystearate is less specific. Signals are obtained for all protons in methyl 12-hydroxystearate, although in some cases several proton signals overlap.

Studies made by previous workers[32] revealed that a single spectrum CSR analysis of a polyfunctional molecule is not possible. Unambiguous assignment of overlapping protons signals can be

accomplished only through the use of several complementary interpretive techniques including an incremental addition study, the construction of proton plots and the calculation of induced shift ratios.

Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Spectroscopy

In ^{1}H NMR (PMR) spectroscopy, the investigator examines signals which are associated with hydrogen atoms and give information about the environment of those hydrogens. In a variation of NMR spectroscopy developed more recently -- carbon-13 nuclear magnetic resonance (^{13}C NMR) -- peaks due to carbon atoms are recorded instead. This relatively new form of NMR is already being applied to fatty acids, and promises to be a powerful tool[33-35].

The multiplicity of ^{13}C NMR signals is determined primarily by the number of protons attached to the carbon under consideration; those with no protons attached, e.g. in carbonyl groups, appear as singlets. In many ^{13}C NMR spectra, a hopeless profusion of overlapping signals is displayed without application of a technique called proton decoupling; the procedure collapses all multiplets to singlets so that the spectrum is greatly simplified[36,37].

Tulloch et al.[58] have assigned chemical shifts to all the separate signals in the ^{13}C NMR spectra of methyl stearate, oleate, and petroselinate by means of the second and third atom isotope effects in the spectra of specifically deuterated esters. All the isomeric oxostearates and most of the hydroxy- and acetoxystearates can be distinguished and identified by their ^{13}C spectra. Rus et al.[59,60] have studied ^{13}C NMR of methyl, methylene, and carbonyl carbon atoms of methyl alkenoates and alkynoates, and double and triple bond carbon atoms of unsaturated fatty acid methyl esters. Gunstone et al.[61,62] have made ^{13}C NMR studies of acetylenic and olefinic fatty acids and esters. Recently, Smith, Jr.[63] has studied the ^{1}H-decoupled spectrum of a conjugated acetylenic PUFA, methyl isanolate. Most recently the carbon -13 pulse Fourier transform NMR technique for measurement of intact plant tissue has been used by Chen et al.[64] for the characterization and estimation of fatty acid composition in seeds of Leucas cephalotus, Stocksia brahuica, and Avena fatua.

Mass Spectrometry (MS)

In recent years mass spectrometry has been widely accepted as one of the most valuable and powerful techniques available to the organic chemist for the structure determination of an ever-increasing variety of natural products. Within these areas, fatty acid esters occupied a unique position in that they represent one of the earliest and most comprehensively studied classes of natural products to be investigated. The use of mass spectrometry for determining the structure of fatty acids has been reviewed by McCloskey[65], Zeman and Scharmann[66] and Klein[67].

The successful mass spectral analysis of glycerides and their derivatives was coincident with the introduction of direct insertion techniques leading to the analysis of triglyceride mixtures[68,69]. Combined gas chromatography and mass spectrometry (GC-MS), associated with refinements in the design of various types of molecular spectra[70,71], has been applied to the analysis of mixtures of fatty acid esters[72,73]. More recent studies of the mass spectra of highly polar lipids such as glycerophospholipids, sphingophospholipids and glycolipids have used a wide range of techniques including 'Soft' ionisation methods to limit the fragmentation of the molecular, or quasi-molecular ion.

Apart from low resolution mass spectrometry (R ≅ 1000) which is the sine qua non of the analytical approach, more specialized techniques include (i) high resolution mass spectrometry (R ≅ 10000) for the accurate measurement of ionic mass - to charge ratio, (ii) specific labelling with stable isotopes, or with functional groups designed to direct fragmentation, (iii) reduction of the electron beam energy in order to limit fragmentation, (iv) metastable ion techniques for the elucidation of specific pathways, and (v) the measurements of ionic appearance potentials yielding thermochemical data.

"Field desorption MS" technique developed by Beckey et al.[74] and Robertson and Co-worker [75] has been used to obtain a greatly different mass spectrum consisting almost entirely of the molecular ion peak.

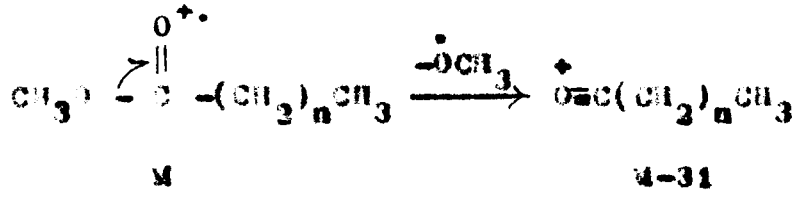
Mass Spectra of Fatty Acid Esters

Most of the mass spectrometric structure work on fatty acids has been performed on the corresponding (usually metayl) esters. Most fragmentation reactions can be classified as either simple cleavage or rearrangements.

The molecular ion (M^+) and M-31

The relative abundance of M^+ increases from methyl pentanoate onwards and its presence can be verified by the

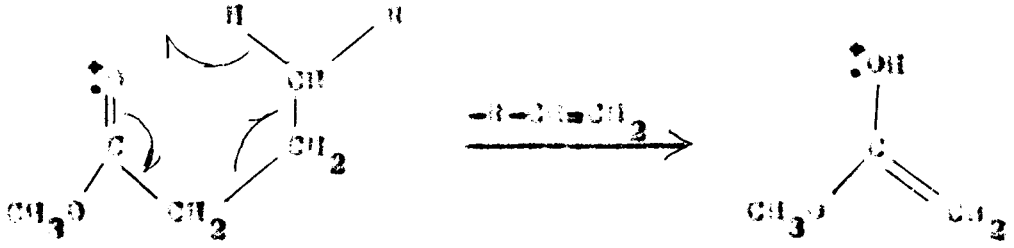
acylium ion, M-31, formed by simple α-cleavage. This peak is of excellent diagnostic value of esters since it is characteristic of methoxyl group in methyl esters.



M M-31

Mass 74

Gamma hydrogen migration to a double bond followed by beta cleavage yields the ion 74 (McLafferty rearrangement)[76] which is the base peak

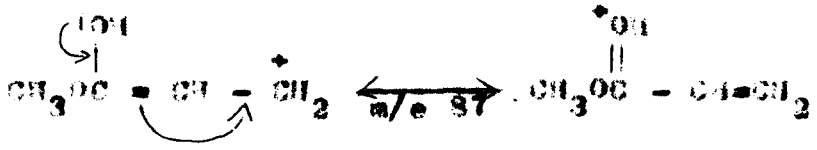


m/e 74

Mass 74 will shift to correspondingly higher masses if C(2) is substituted. Mass 75 is usually observed to be more abundant than required by the isotope peak of m/e 74. Most of the observed m/e 75 peak is due to protonated form of m/e 74. The origin of second transferred hydrogen is not known but is apparently abstracted randomly from the chain.

Oxygen-containing ions $[(CH_2)_nCOOCH_3]^+$

These ions are arithmetically found at m/e (59 + 14n), i.e., m/e 87, 101, 115, 129, 143, 157 etc. The lowest potential member, m/e 73, is essentially absent, probably owing to the unfavourable location of a positive charge adjacent to a positively polarised carbonyl group. The most abundant member, m/e 87, derives its stability from the enol form.



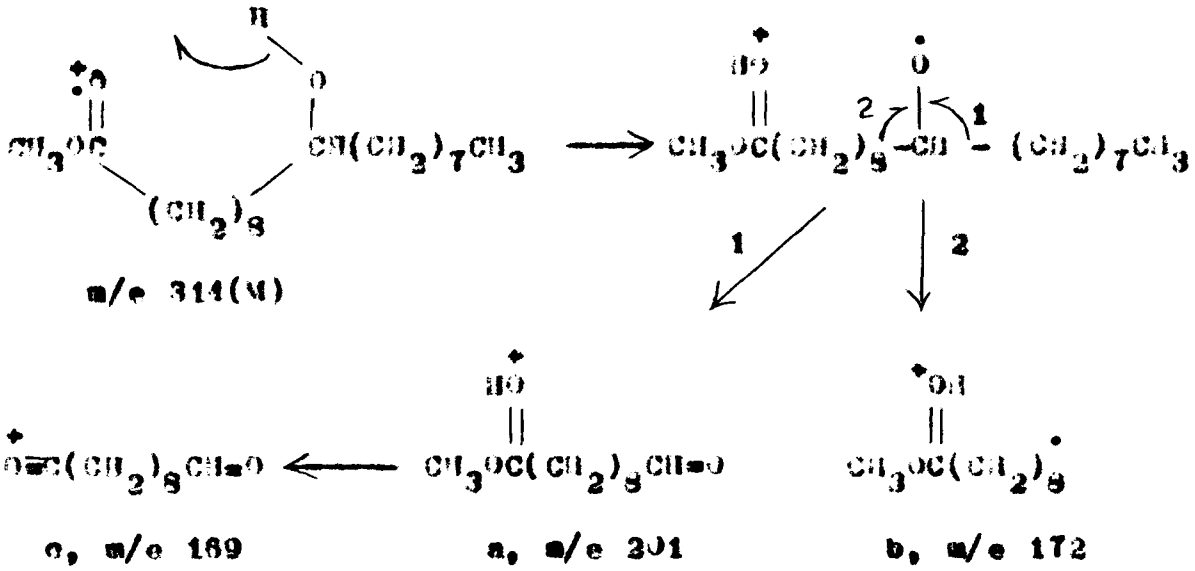
Hydrocarbon ions

Both simple cleavage and rearrangement processes contribute to the formation of hydrocarbon ions, the most prominent of which (m/e 69, 83, 97 etc.) are from the saturated series C_nH_{2n+1}. The presence of hydrocarbon ions in the mass spectrum in general serves no structural purpose, but may occasionally be helpful in establishing a reference point for counting the spectrum.

Hydroxy fatty esters

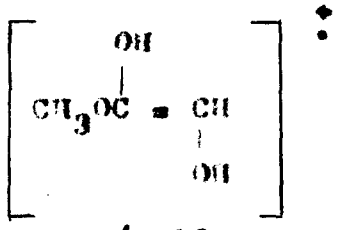
In the mass spectra of hydroxy esters it has been observed that the molecular ion peak is usually small or absent. Taking an example of DL-10-hydroxyoctadecanoate[77] the peaks at m/e M-32 (loss of methanol) and peaks at m/e 264, 232 and 190 characteristi

for methyl oleate indicate that some dehydration has occurred. The location of the hydroxyl group is indicated by the base peak at m/e 201, the ion arising through α-cleavage on the far side of the hydroxyl group. Further loss of methanol from this ion gives the 'ketene'-type ion of m/e 169. Another characteristic feature is the ion of m/e 172 that arises from α-cleavage in the near side of the hydroxyl group with shift of one hydrogen atom from the fragment lost.

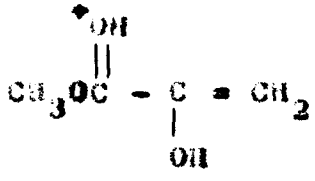


Variation of the position of a functional group in the chain can give rise to considerable change in ion abundances, particularly when the function is moved near either extreme end. For example, the mass spectra of methyl esters containing a hydroxyl group in position 3 are so dominated by m/e 103 (due to the α-cleavage on the far side of the hydroxyl group)

that [illegible] and lower mass range fragment ions are virtually absent. [77,78] If the hydroxyl group is located on the alpha carbon atom, cleavage between C(1) and C(2) is facilitated, leading to loss of the carbomethoxy group. Ions of m/e 90 and 103 correspond, respectively, to the rearranged ion of m/e 74 and the ion of m/e 87 found for saturated methyl esters, the hydroxyl group being retained in the ion.



m/e 90

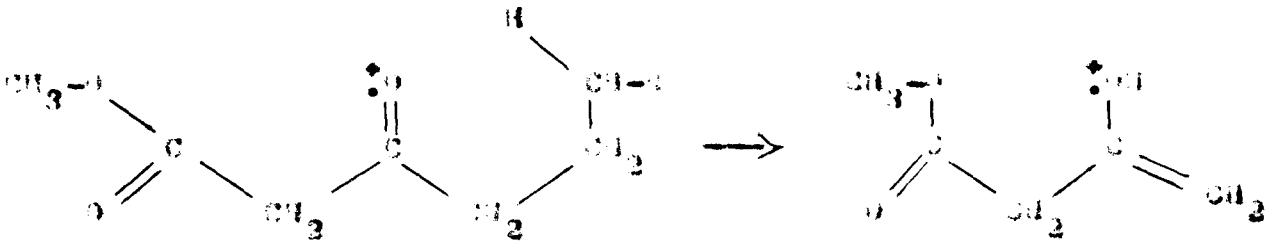


m/e 103

If the hydroxyl is silylated, cleavages on either side of the substituted carbon atom result in prominent ions. For TMS ethers of diols in the series $CH_3(CH_2)_n-CH(OTMS)-CH(OTMS)-(CH_2)_m-CO_2CH_3$, cleavage takes place between the two OTMS groups producing two fragments, $[CH_3(CH_2)_n-CH(OTMS)]^+$ and $[-CH(OTMS)-(CH_2)_nCO_2CH_3]^+$ with the positive charge retained almost equally on both fragments. Silylation of hydroxyl groups in methyl esters of unsaturated hydroxy acids provides compounds that give mass spectra which can be readily interpreted, whereas spectra of underivatized esters are extremely difficult to evaluate. The relationship of the double bond(s) to the trimethylsiloxy (TMS) group results in specific mass spectral patterns.

Keto fatty esters

The position of a keto oxygen atom generally can be deduced easily from the mass spectrum[77] where both alpha and beta cleavages with rearrangement occur. Deviations from the pattern occur when the oxo group is located near the methoxycarbonyl group or near the terminal carbon atom of the hydrocarbon chain. In the 2-oxo compound the tendency to cleavage between the vicinal oxo groups is so strong that the acylium ion of m/e 4-59 dominates the spectrum. In case of methyl 3-oxooctadecanoate the base peak at m/e 116 is due to ions formed by 4,5-cleavage (β to the 3-oxo group) with migration of one hydrogen atom as shown below.

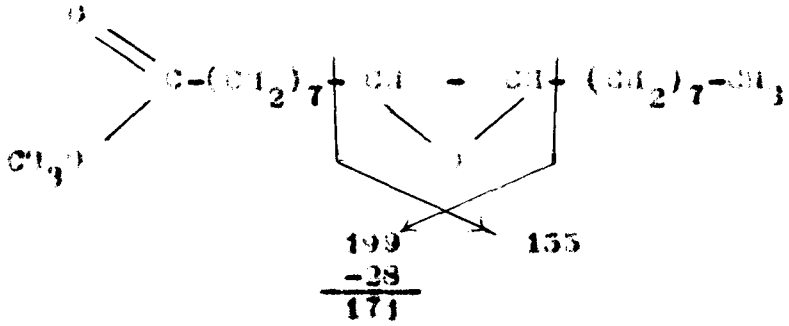


m/e 313 m/e 116

When the oxo group is located near the end of the hydrocarbon chain, the same cleavage pattern is observed except that for positions (-2) and (-1) ions formed by β-cleavage on the hydrocarbon side of the oxo group and rearrangement are absent since no hydrogen atoms at γ-position with respect to the oxo group are available.

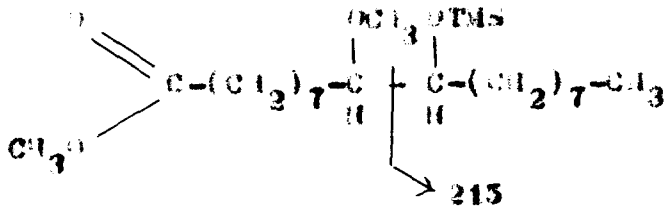
Epoxy fatty esters

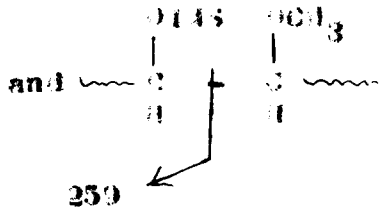
Saturated epoxy esters give mass spectra, the interpretation of which is so straightforward that epoxidation and MS form an established procedure to locate double bonds[79,80]. For example, the spectrum of methyl 9,10-epoxystearate[77] has a base peak of m/e 155 arising from cleavage alpha to the epoxide ring. Cleavage on the other side of the functional group produces a much smaller but significant peak at m/e 199. In contrast, addition of a double bond into the molecule changes the spectrum so radically that assigning the location of the epoxide ring is almost impossible.

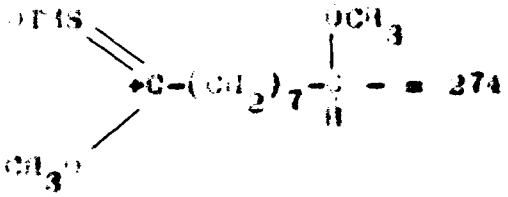


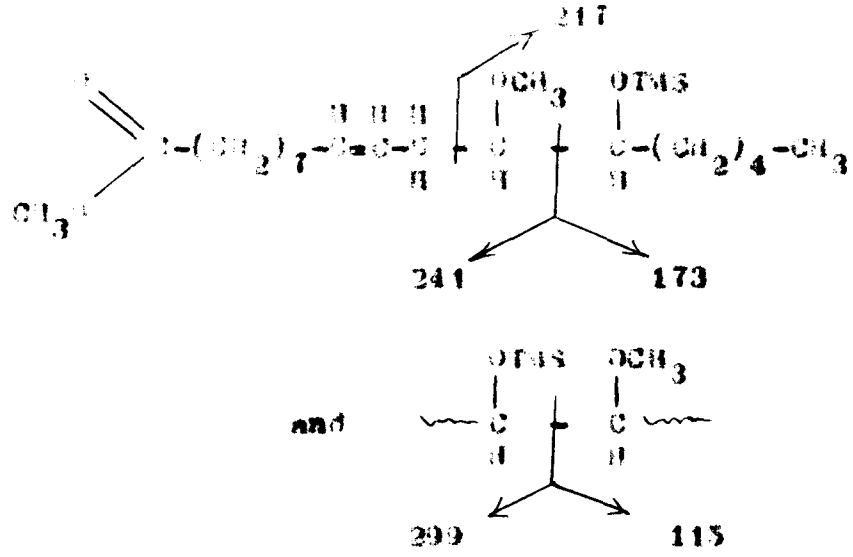
Ten-Cate[81] converted the methyl esters of the epoxy acids into the cyclopentanone-ketals in the presence of traces of BF_3 and studied their mass spectra to confirm the structure of epoxy acids. Later on, Kleiman and Spencer[82] have studied the mass spectra of epoxy esters by first converting the epoxide to a methoxy-hydroxy group (by treatment with BF_3/MeOH) and then silylating. In esters without a double bond one methylene

unit from the oxygenated site, two peaks define the location of the oxygenated group. Both peaks arise from cleavage between the carbon atoms containing the methoxyl and the siloxyl groups. The significant ions contain the siloxyl groups and not the methoxyl groups. In esters that have a methylene group separating a double bond from the methoxy-trimethylsiloxyl groups, they also have the ions described above plus an ion from α-cleavage on the side of the methoxy-trimethylsilyloxy substituent closet to the double bond.









Unsaturated fatty acid esters

The location of double bonds in fatty acids by mass spectrometry has been approached in many ways which have been summarized in reviews[61-67,84]. Methyl esters of all positional and geometrical isomers of oleic acid (except the α,β-unsaturated) give mass spectra that are practically identical to that of methyl oleate[53]. The spectra of monounsaturated esters are further indistinguishable from cyclopropane esters of the same number of carbon atoms. The problem of double bond location turns on the choice of a suitable derivative yielding distinct and limited fragmentation without migration, often directed by a charge-stabilizing group, such as TMS or a resonance-stabilized ring. Among these methods the procedure of methoxymercuration-demercuration has been reported[33] to be simple, reliable and rapid. Mass spectra of the methoxylated

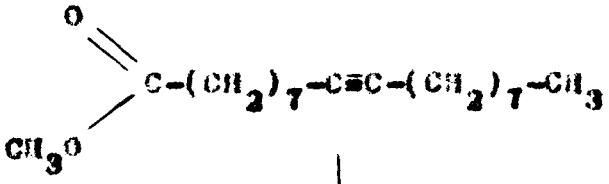
esters are characterized by intense peaks due to cleavage adjacent to methoxy functions which allow the position of the original double bond in the chain to be ascertained. Fragments of the type $R-\overset{+}{C}H-\ddot{O}CH_3 \longleftrightarrow R-CH=\overset{+}{O}CH_3$ are expected to be particularly prominent in the mass spectra of such methoxy esters. A modification of the method, which includes the mass spectrometry of methoxybromo/methoxyiodo derivatives of long-chain unsaturated esters prepared from methoxymercuric acetate adducts, has also been reported[96].

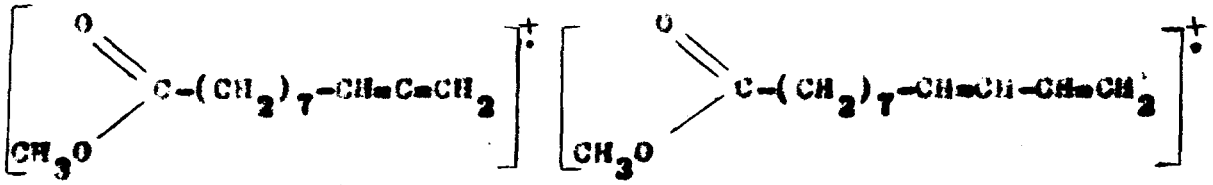
Anderson and coworkers[97,98] have reported a more satisfactory solution to the problem of determining double bond position. These authors have demonstrated that more useful results are obtained with amides and particularly pyrrolidides from the mass spectra of which double bond position can be deduced directly. The spectra of the octadecenylpyrrolidides contain clusters of peaks from the polar part of the molecule. If an interval of 12 atomic mass units, instead of the regular 14, is observed between the most intense peak of each cluster of fragments containing n and n-1 carbon atoms in the acid moiety then a double bond occurs between carbon atoms n and n+1 in the molecule. The rule is valid for the double bonds occurring at positions C-5 to C-15 in an 18-carbon chain and has been applied to acids having 19-24 carbon atoms. Anderson *et al.*[99] have also applied the derivatization for mass spectrometric

determination of double bond positions in polyunsaturated fatty acids. Plattner et al.[90] have developed a rapid micro-procedure to locate double bonds in polyenoic fatty esters containing from one to four double bonds through partial oxymercuration.

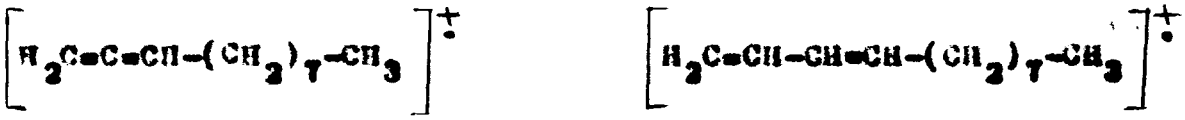
Acetylenic fatty acid methyl esters

Recently Kleiman et al.[91] analyzed an almost complete series of methyl octadecynoate (all but the 3,4 and 16,17 isomers) by mass spectrometry. The basic mass spectral pattern is one of cleavage with McLafferty rearrangement either of the acetylenic band or of the isomeric allenes found by rearrangement. For example, the mass spectrum of methyl octadec-9-ynoate (methyl stearolate) showed the following four characteristic fragment ions:





m/e 196 (A) m/e 210 (B)



m/e 152 (C) m/e 166 (D)

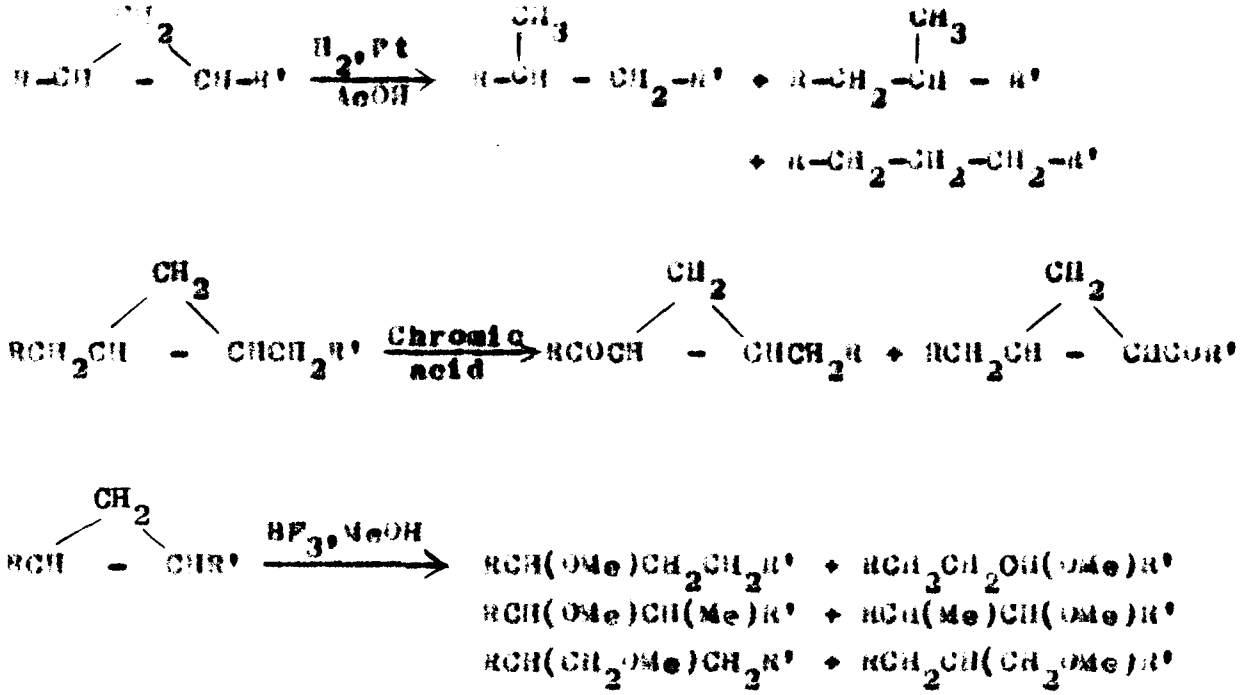
Ions with 32 mass units (CH_3OH) less than ions A and B were also present. Ions containing the terminal part of the molecule (C and D) are found most abundant when the triple band is close to this part of the molecule. Fragment ions A and A-32 are the most intense of the characteristic ions when the acetylenic bond is near the ester function.

Oxymercuration of acetylenic esters produces both isomeric oxoesters. Addition of excess of $NaBH_4$ resulted in a mixture of hydroxy esters. The major ions were determined for the silylated hydroxy esters formed from each member of the series. Each derivatized acetylenic ester produces upto four major and two minor characteristic peaks.

Cyclopropane and Cyclopropene fatty acid esters

Cyclopropane fatty acids give mass spectra that are practically indistinguishable from those of the corresponding unsaturated isomers.

More usefully, the cyclopropane system may be fixed by some chemical reactions leading to a product, or more usually a mixture of products, which are identified by mass spectrometry. These reactions include hydrogenolysis[92], oxidation[93] and reaction with methanolic borontrifluoride.[94]

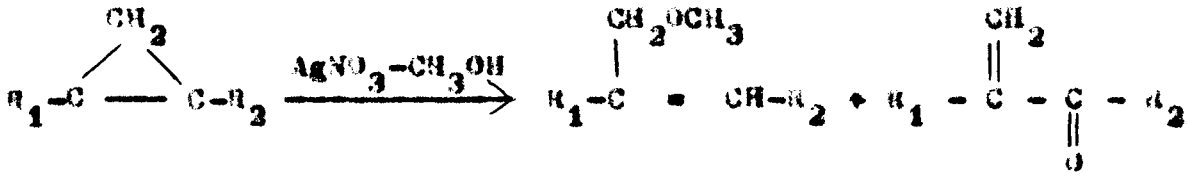


Recently, Gensler and Marshall[95] have reported the structure determination of cyclopropane-substituted acids by mass spectrometry. Chromium oxidation of cyclopropane fatty esters converts the alkyl methylene group next to the three-membered ring to an oxo group.

Cyclopropene esters are apparently too labile to be subjected directly to mass spectrometry, but the position of ring can be located by this technique if the compound is oxidized to a β-diketone[96] or reacted with methanethiol to form a product with the sulphur atom attached to either of the ring carbons[97].

Eisele *et al.*[98] have studied the mass spectral fragmentation pattern of the silver nitrate-methanol treated

derivatives of a number of cyclopropenoid compounds.



All the cyclopropene silver nitrate derivatives show a base peak of m/e 95, probable structure $C_8H_{13}^+$ or $C_5H_9O^+$. Ions at m/e 41, 43, 55, 71, 81 and 95 are intense in all spectra. Also, each methoxy derivative shows an ion equivalent to the loss of R_1 from the parent ion. A characteristic parent minus 32 mass, which would indicate the probable loss of methanol from the parent ion, is present in all spectra.

Other large ring cyclic fatty acids such as those with cyclopentene[99], cyclohexene[100] or furanoid[101] give quite distinct spectra from which the structures are readily deduced.

B. PRESENT WORK

Although addition of nitrosyl chloride to olefinic substrates has been studied[3,4,31,32,34] nitrosochlorination of unsaturated fatty acids has received only limited study. The reported conversion of nitrosochloro derivatives to the valuable aziridines[34] has in recent years highlighted the application of this reaction to fatty acid chemistry. The preparation of a variety of new fatty acid derivatives from internal, terminal and α,β-unsaturated acids has been a subject of recent contributions[102-108] from the author's laboratory. As a part of our continuing study of the derivatization of aliphatic compounds related to fats, the nitrosochlorination of olefinic fatty acids was taken up for the present study.

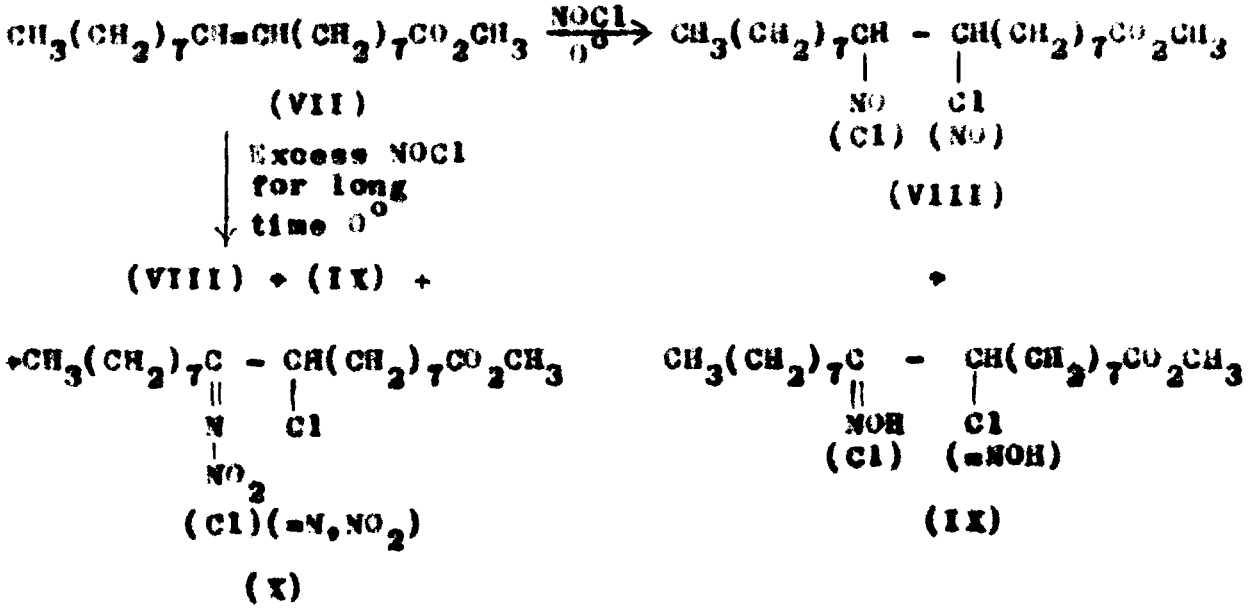
Nitrosochlorination of methyl oleate (VII)

The nitrosochlorination of oleic acid was first carried out by Tilden *et al.* in 1894.[18] Re-examination by Miller *et al.*[19] has demonstrated that the addition of nitrosyl chloride to methyl oleate is essentially quantitative, when the reaction is conducted at 2° and the solvent is methylene chloride. However, no attempts were made to isolate and characterise the individual products of the reaction. Further, reports of the formation of anomalous products during the recent studies of NOCl reaction upon olefinic compounds led the author to carry out the present investigation.

The various products formed during the reaction of NOCl upon methyl oleate (Scheme 1) under varying conditions were isolated and characterized with the aid of chromatographic and spectroscopic techniques.

Methyl oleate (VII) on treatment with approximately stoichiometric quantities of nitrosyl chloride <u>in situ</u> (<u>iso</u>-amyl nitrite + HCl) at 0° in methylene chloride solution for 3-4 hr gave essentially quantitative yield of (VIII) an azure oil, in admixture with a little of its isomeric oximino form (IX). After usual work-up of the reaction mixture, there was obtained

<u>Scheme 1</u>



a blue liquid. Reaction products were separated into two fractions (1 and 2) by column chromatography over silica gel. A major fraction 1, eluted first, as a blue coloured liquid contained chiefly a nitrosyl chloride adduct (VIII) in admixture with a little oximino form (IX). With respect to the positions of the nitroso and the chlorine, product (VIII) is probably a mixture of isomers [methyl 9(10)-chloro-10(9)-nitrosostearate, (VIII)]. That both isomers were indeed formed was evident from the TLC of the reaction product which showed two closely associated spots (Rf 0.9 and 0.85). A minor fraction 2, of lower Rf (0.24), was characterized as the oximino form(IX) of compound (VIII). This fraction was also believed to be an isomeric mixture of oximes [methyl 9(10)-chloro-10(9)-oximinostearate, (IX)]. The characterization of different fractions was made on the basis of microanalysis, IR and NMR.

Characterization of Fraction 1

The product was tested qualitatively for the presence of halogen by the Beilstein test. It gave satisfactory microchemical analysis for $C_{19}H_{36}NO_3Cl$ (Compound (VIII/IX). The IR spectrum (Fig.1, Sheet 1) of Fraction 1 showed, besides the bands usually found in long-chain fatty esters, absorption at 1570 (N=O), 1110 (C-N), and 710 (C-Cl) cm^{-1} indicative of the nitrosochloride functions. The presence of weak bands at

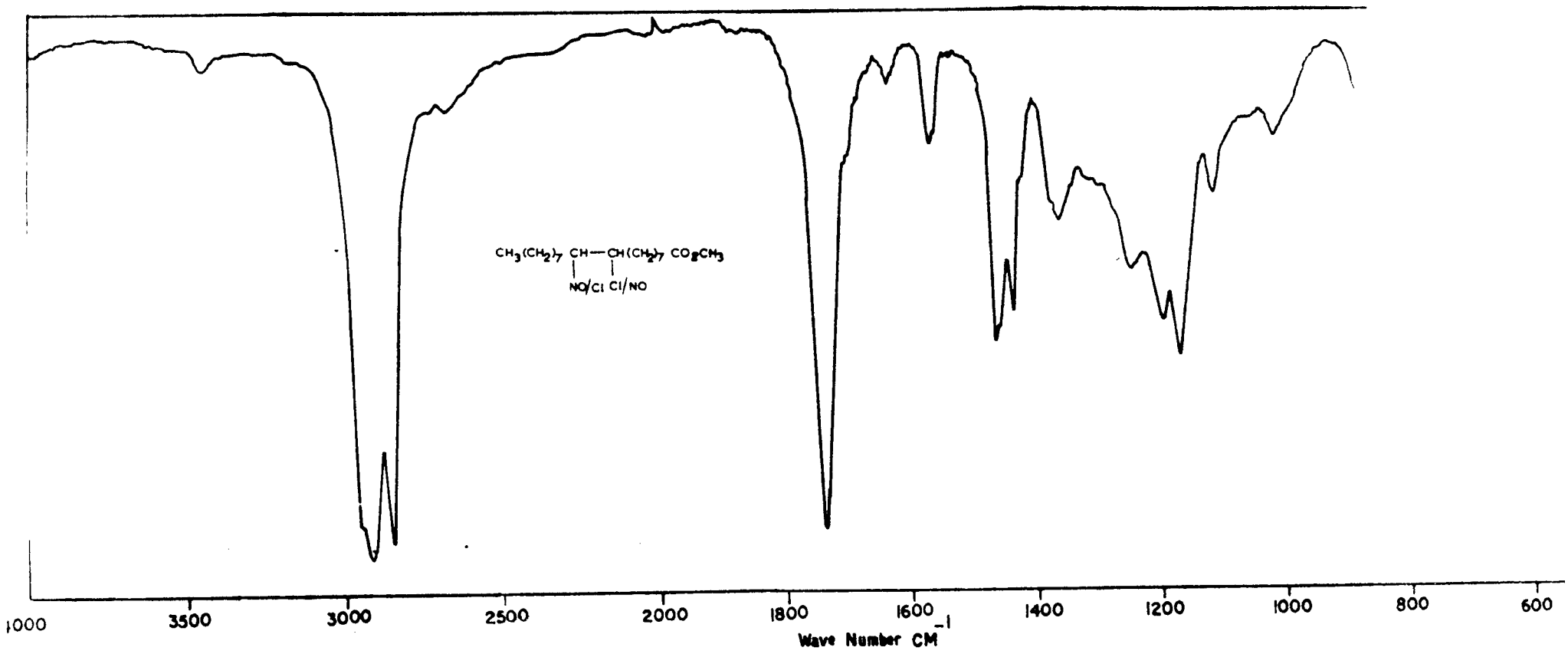


Fig. 1. IR spectrum of methyl 9(10)-chloro-10(9)-nitrosooctadecanoate (VIII)

1640 (C=N), and 3450 (OH) cm^{-1} indicated the presence of ketoxime, a rearranged product of (VIII) in minor amount. The NMR spectrum (Fig.2,Sheet II) was decisive in arriving at a more firm conclusion regarding the composition of fraction 1 as an isomeric mixture of methyl 9(10)-chloro-10(9)-nitrosostearate (VIII) and methyl 9(10)-chloro-10(9)-oximinostearate (IX), former being in a major amount. NMR spectrum displayed a signal at τ 2.52 (D_2O exchangeable) ascribed to the oximino group proton (=N-O<u>H</u>), an unresolved multiplet at τ 6.12 for methine proton adjacent to the chlorine atom and an unresolved multiplet centred at τ 8.65 assigned to the methine proton adjacent to the nitroso group. A signal at τ 8.38 was also observed due to methylene protons α to the -CHCl-$\left[-CHCl-C\underline{H}_2-\right]$. Other proton signals usually present in fatty acid ester at τ 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OC\underline{H}_3$), 7.76 (2H, protons α to ester group), 8.65 (br s, chain methylene protons), and 9.12 (distorted t, 3H, terminal methyl protons) were also observed.

<u>Characterization of Fraction 2</u>

Microanalysis of Fraction 2 (compound IX) supported the formula $C_{19}H_{36}NO_3Cl$ (positive Beilstein test). IR spectrum of the compound (IX) displayed bands at 3450 (OH), and 1640 (C=N) cm^{-1} indicative of the oximino group. NMR spectrum (Fig.3,Sheet III) showed an apparent multiplet centred at τ 2.65 for one proton which is D_2O exchangeable and assigned to the hydroxyl proton of oximino group (=N-O<u>H</u>). Another signal at τ 6.1 is attributed to the methine proton adjacent to the Cl atom (-C<u>H</u>Cl-). Other signals characteristic for long-chain fatty acid ester were also exhibited:

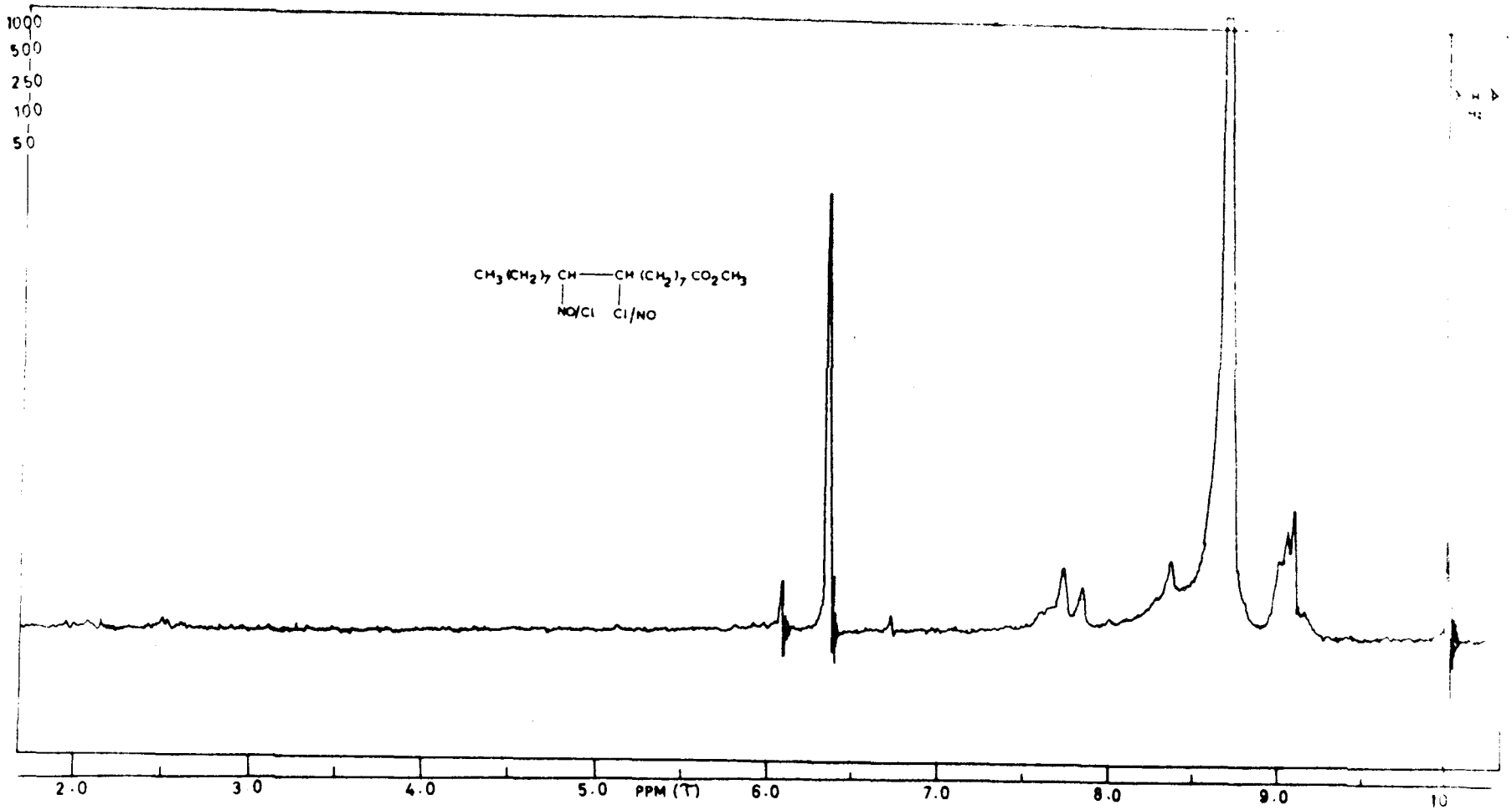


Fig.3. NMR spectrum of compound (VIII)

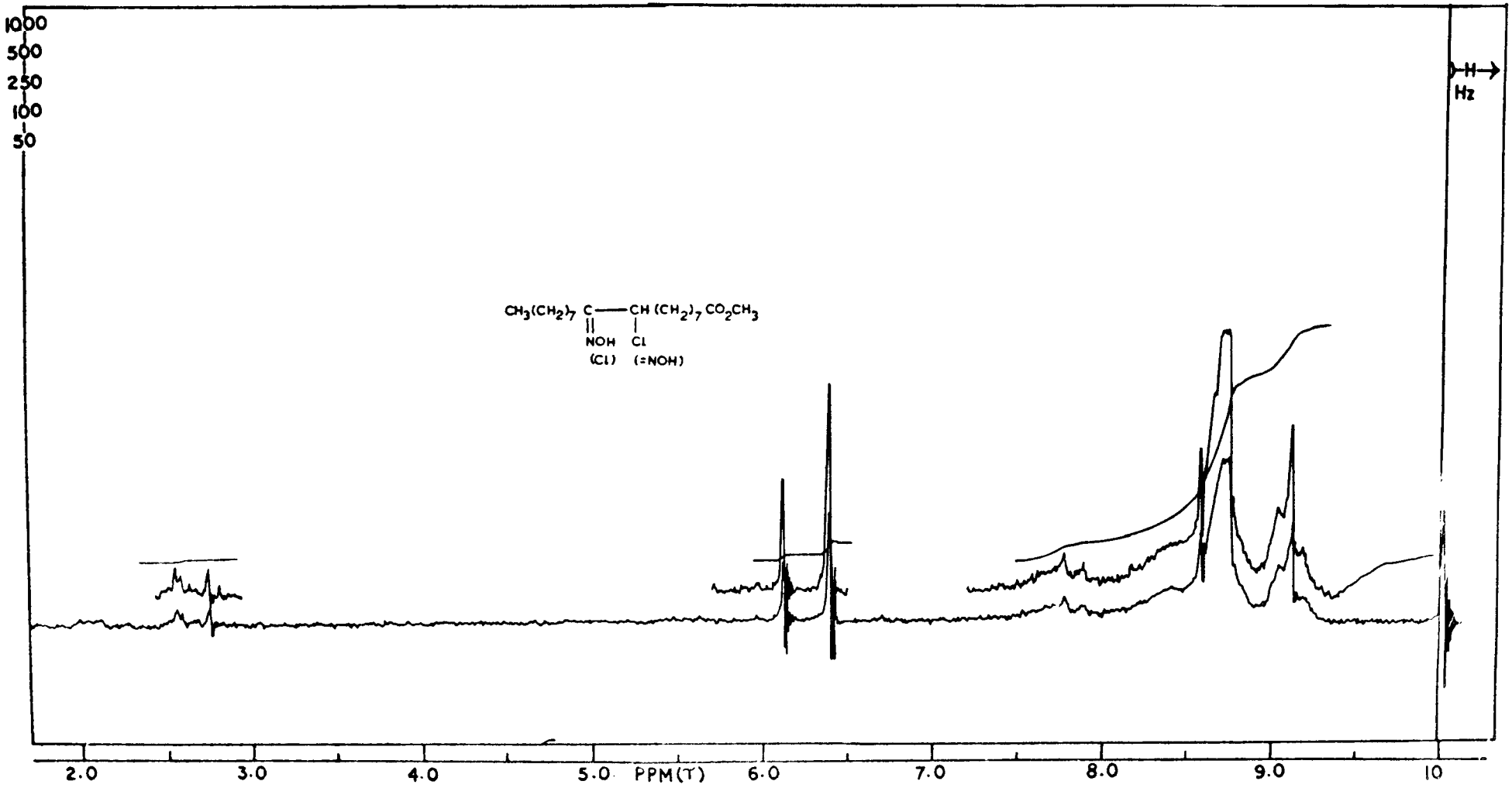


Fig.3. NMR spectrum of methyl 9(10)-chloro-10(9)-oximinooctadecanoate (IX)

the ester methyl protons ($-\overset{O}{\overset{\|}{C}}-OCH_3$) gave rise to a singlet at τ 6.35, protons α to the ester carbonyl gave rise to a triplet at τ 7.76, the methylene protons appeared as a large broad signal centered at τ 8.65. A distorted triplet appeared at τ 9.12 for the terminal methyl group. Both IR and NMR substantiated the assigned structure(IX).

Presence of hydrogen on the carbon carrying nitrosyl group permits rearrangement to the oxime[3]. For most of the examples cited in the literature this rearrangement is spontaneous or is promoted under the mildest conditions. The product chlorooxime is usually a solid, more stable than the nitroso isomer. But in this case isomerization seems to be very slow. About 0.5-1.0% of oxime was already present in the freshly prepared compound (VIII) as evidenced by TLC, IR and NMR. Oxime content increased at room temperature to a maximum of about 20% after about two weeks. As regards the formation of oxime our observations conform to those reported by Miller et al.[19]

Many nitrosochloro compounds dimerize to white solids[3]. We have found no evidence for appreciable dimerization. The persistent blue colour at 0-5° and the presence of the strong IR nitrosyl band as well as nonformation of any solid adduct, all indicate little, if any, dimer formation. Miller et al.[19] also did not report the formation of dimer during nitrosochlorination of methyl oleate. Precedents exist for the suggestion

that dimer formation is inhibited due to steric hindrance.

By treating with an excess of NOCl for a long time methyl oleate gave a product (X, 10%) in addition to products (VIII) and (IX). The product (X), having Rf value greater than the Rf of the oxime (IX) was characterized as methyl 9(10)-chloro-10(9)-nitriminostearate on the basis of microanalysis, IR and NMR.

Characterisation of product (X)

Strong IR bands at 1550 and 1360 (NO_2) cm^{-1} and a medium band at 1640 (C=N) cm^{-1} characteristic of nitrimines were observed. NMR spectroscopy (Fig.4, Sheet IV) was useful in confirming the structure of product (X). In addition to expected signals for remainder of the molecule (τ 6.34, 7.78, 8.67, and 9.12), diagnostically useful signals were observed at τ 5.86 (mc) due to the methine proton adjacent to Cl atom (-CHCl-) and at τ 7.38 (t) for methylene group α to the nitrimino group $\left[-CH_2-C(=N.NO_2)-\right]$.

The nitrimine (X) is formed by the oxidising action of NOCl upon oxime (IX). The oxidizing action of NOCl to convert an oxime into nitrimine was first reported by Shiue et al.[24] and later confirmed by other workers[30,34]. The mechanism of nitrimine formation (eq. 10) suggested by Freeman[25,26] and supported by Boswell[27] seems adequate to account for the results obtained in this reaction.

Sheet - IV

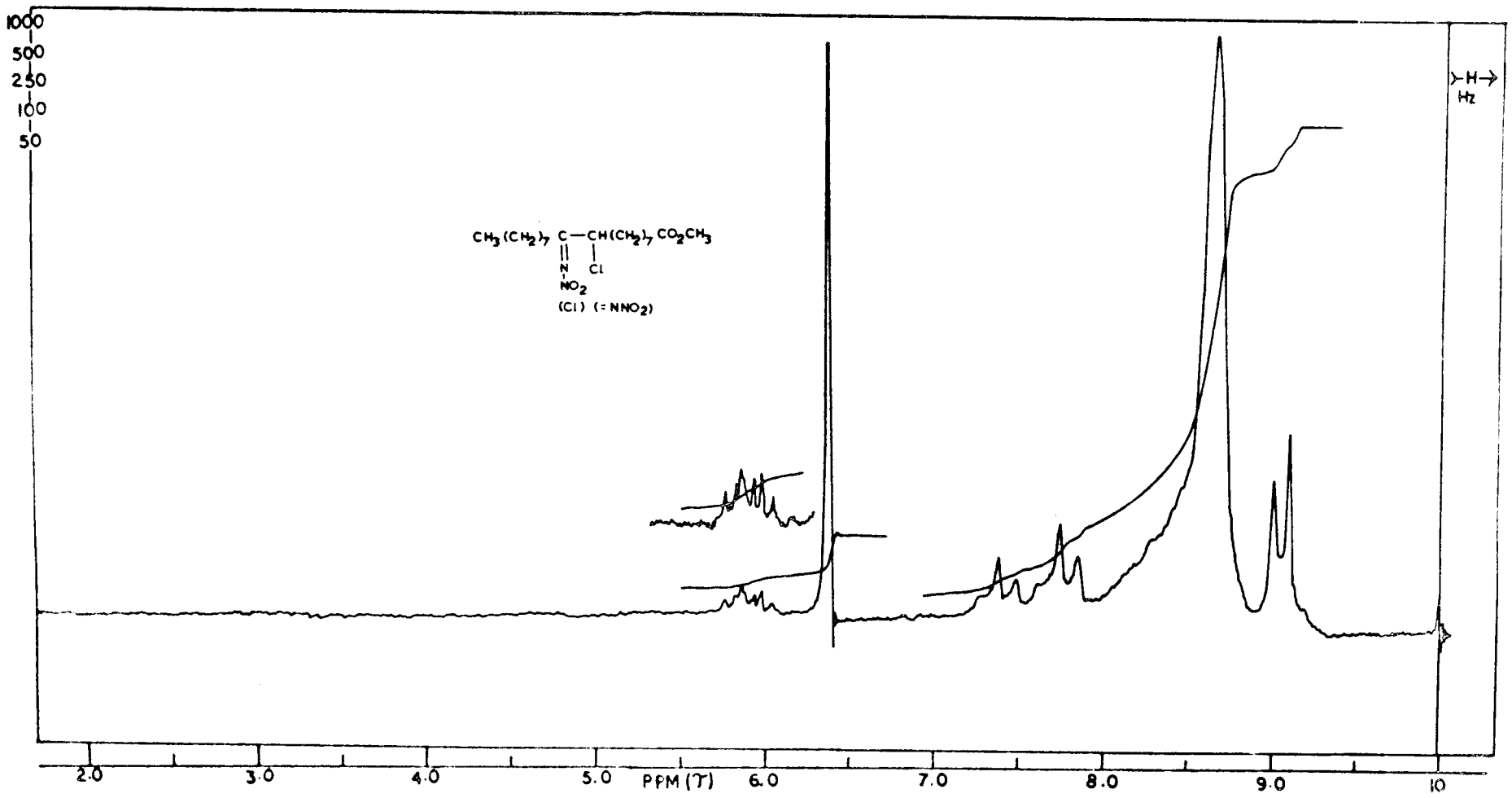


Fig.4. NMR spectrum of 9(10)-chloro-10(9)-nitriminooctadecanoate (X)

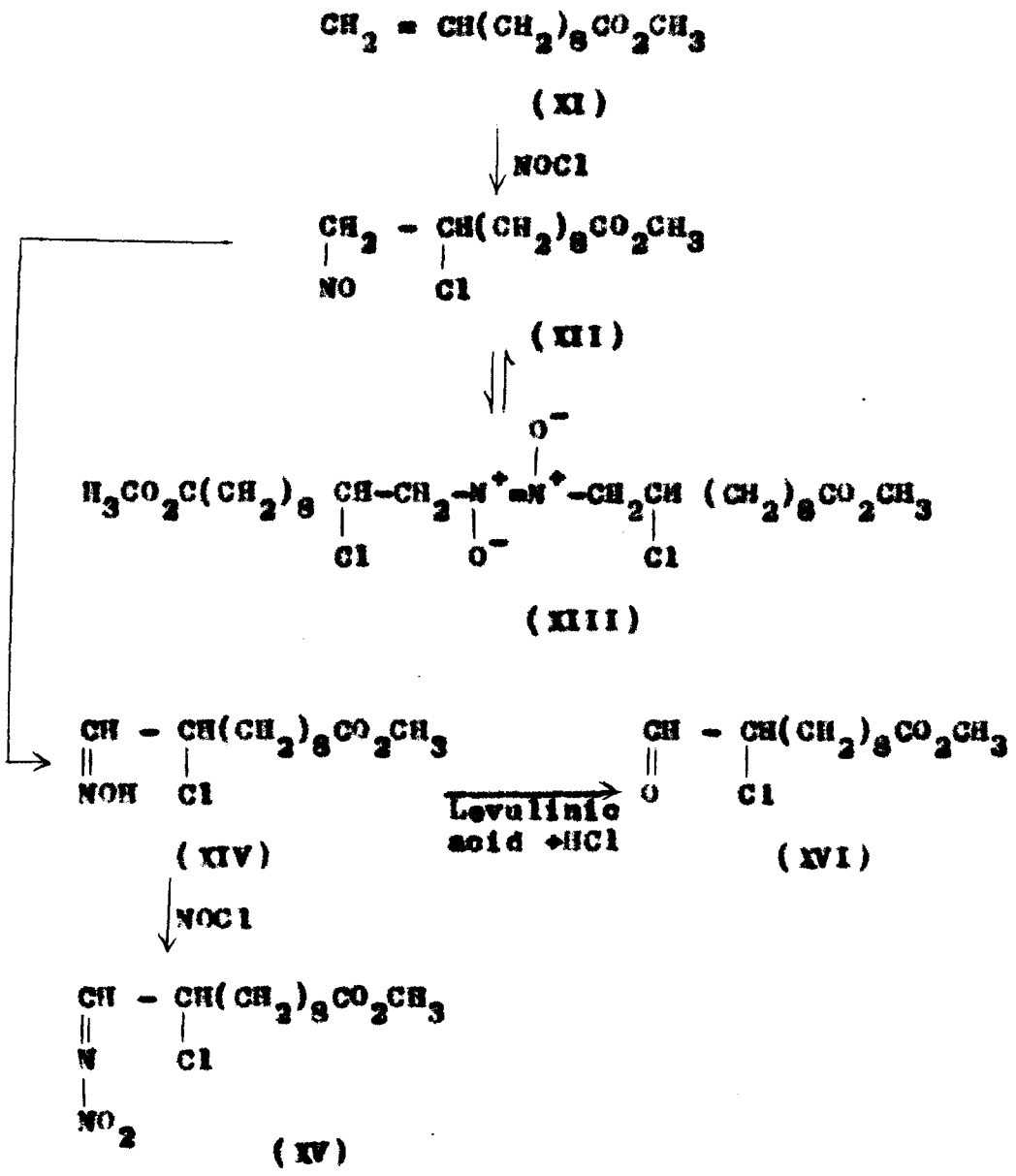
The formation of nitrimine was not reported by Miller *et al.*[19] However, they observed that when methylene chloride solution of compound VIII was treated with NOCl for a long time, infrared showed the presence of nitro group in the product. From the foregoing results it is surmised that the nitro band is due to the formation of nitrimine which has been isolated and characterised in the present study.

Nitrosochlorination of methyl 10-undecenoate (XI)

The study of the reactions of 10-undecenoic acid is interesting in fat chemistry due to a variety of reasons. The unique feature of 10-undecenoic acid is the presence of a terminal double bond. In order to probe the regioselectivity of nitrosyl chloride addition on an unsymmetrically substituted olefinic fatty acid, methyl ester of 10-undecenoic acid, XI, was selected as a model substrate for nitrosochlorination reaction.

Reaction of methyl 10-undecenoate (XI) with NOCl *in situ*, resulted in the formation of four distinct products (XII-XV) as evidenced by analytical TLC. These components were separated by silica gel column chromatography. Formation of a chloro nitroso product (XII) was indicated by the appearance of a bluish green colour in the reaction mixture. IR spectrum of the product also revealed the formation of nitrosyl chloride adduct. Work-up of the reaction mixture yielded no appreciable amount of the adduct in the pure form as it easily dimerizes or rearranges to an oxime.

Scheme - 2


CH2 = CH(CH2)8CO2CH3
(XI)
NOCl
CH2 - CH(CH2)8CO2CH3
NO Cl
(XII)
O−
H3CO2C(CH2)8 CH-CH2-N+=N+-CH2CH (CH2)8CO2CH3
Cl O− Cl
(XIII)
CH - CH(CH2)8CO2CH3
NOH Cl
(XIV)
Levulinic acid +HCl
CH - CH(CH2)8CO2CH3
O Cl
(XVI)
NOCl
CH - CH(CH2)8CO2CH3
N Cl
NO2
(XV)

Characterisation of the compound (XIII)

The product (XIII) separated as white solid (mp 95°), gave satisfactory microanalysis for $(C_{12}H_{22}O_3NCl)_2$. The molecular mass determination by Rast method[109] in camphor supported the molecular formula $(C_{12}H_{22}O_3NCl)_2$ for compound (XIII). It gave positive Beilstein test. The IR spectrum (in nujol) showed, besides the bands usually found in long-chain fatty esters absorption at 1370 cm^{-1} indicative of dimer formation (Fig. 5, Sheet V). The absence of nitrosyl band in the region 1520-1570 cm^{-1} further supported the dimer formation. The NMR spectrum (Fig.6, Sheet VI) also supported the structure of compound (XIII) as dimer of methyl 10-chloro-11-nitrosoundecanoate. The NMR spectrum exhibited the significant signal at τ 5.42 for six protons due to the methine protons adjacent to chlorine atom and methylene groups adjacent to nitrogen ($-\underline{CH}_2-\overset{O^-}{\underset{|}{N^+}}=$). Other usual fatty ester signals were observed at τ 6.34 (s, 6H, ester methyl), 7.77 (protons α to the ester $-\overset{O}{\overset{\|}{C}}-$ group) and 8.67 (br s, shielded chain methylenes). The dimer (XIII) appears to have a trans structure as suggested by Gowenlock and Luttke[7] in their IR spectral studies on dimers of nitroso compounds.

Characterisation of the compound (XIV)

The compound (XIV) was separated as a white solid (mp 42°) in pure form having R_f 0.2. It responded to Beilstein test. The compound (XIV) was analysed for $C_{12}H_{22}O_3NCl$. Its IR spectrum

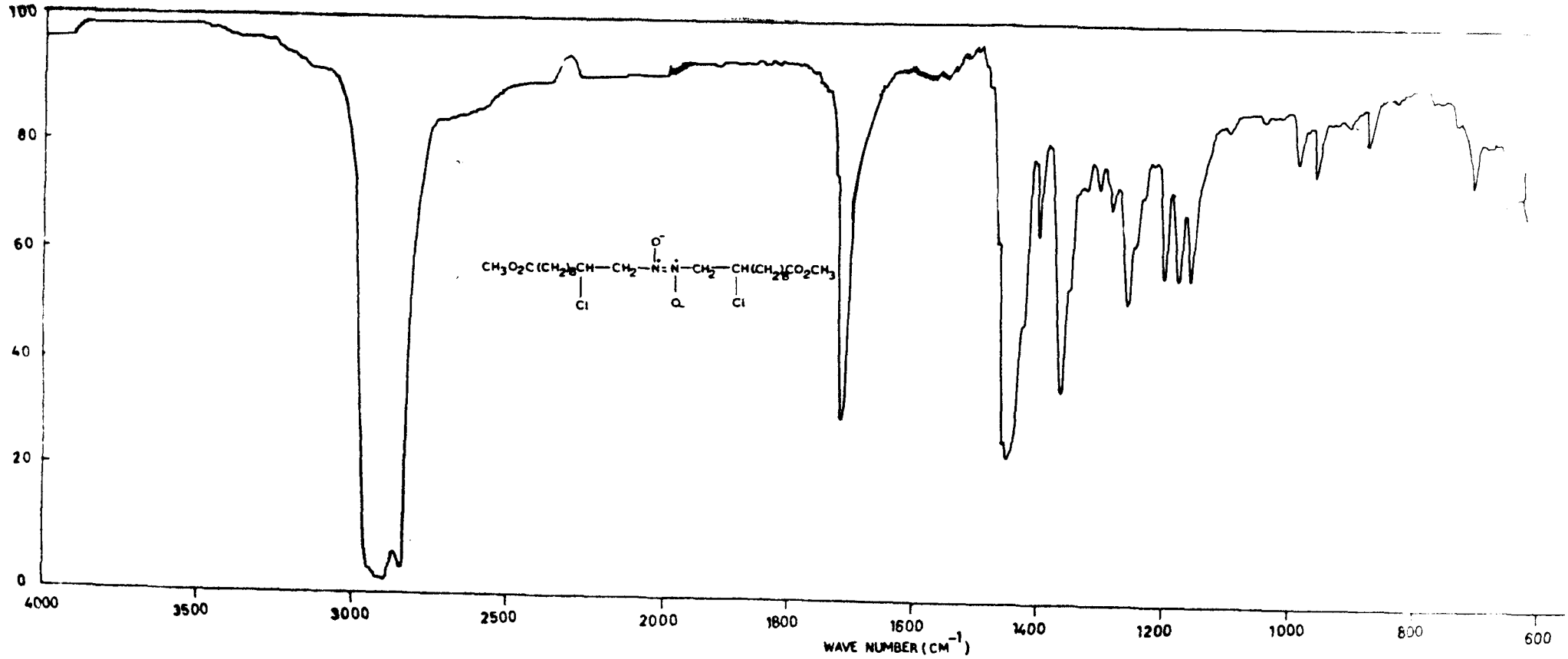


Fig.5. IR spectrum of dimer of methyl 10-chloro-11-nitrosoundecanoate (XIII)

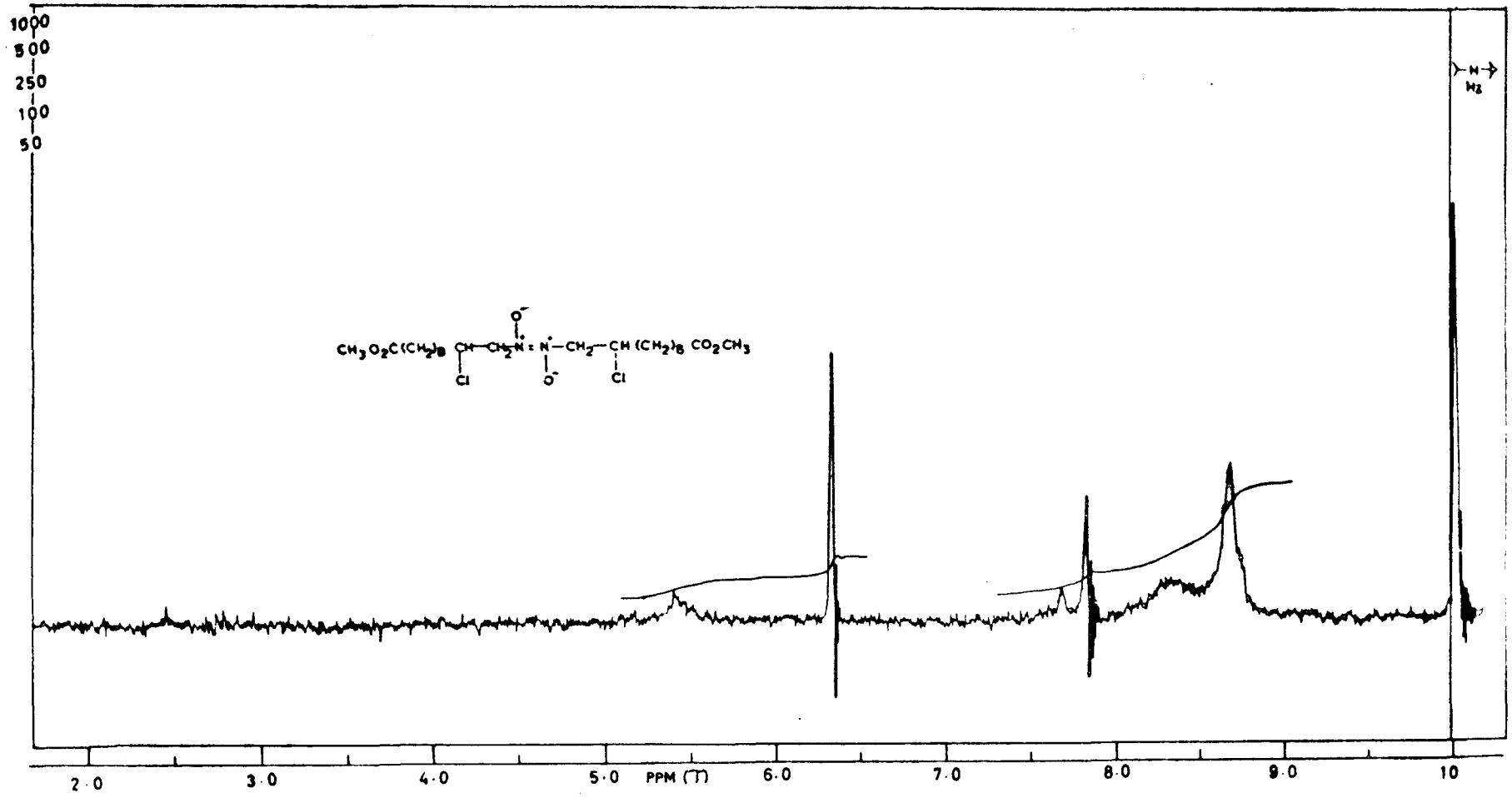


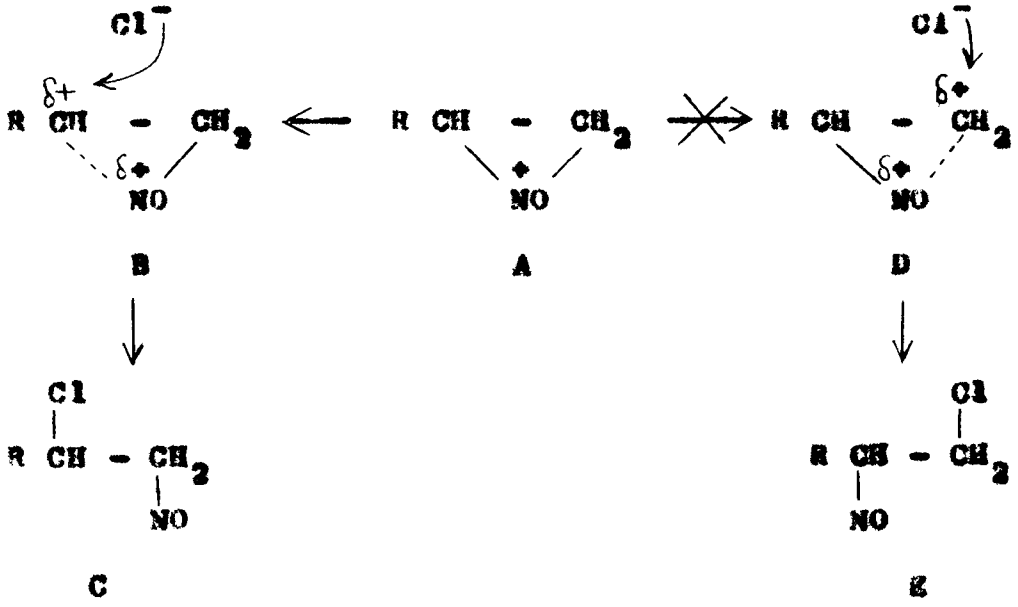
Fig.6. NMR spectrum of compound (XIII)

gave absorption at 3300 (OH) and 1690 (C=N) cm^{-1} attributed to the oximino group and at 730 cm^{-1} to C-Cl linkage. Its NMR spectrum was more informative regarding the position of oximino group in the fatty acid chain. It exhibited an apparent singlet at τ 2.6 which can be assigned to the proton of oximino group (=N-OH). The proton was found to be exchangeable with deuterium. The proton at C-11 appeared at τ 3.6 (-CH=NOH), which conclusively proves the attachment of oximino group to the terminal carbon atom (C-11). Methine proton adjacent to chlorine atom displayed a signal at τ 5.66. Other NMR signals were observed at τ 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 7.76 (2H, α to the ester $-\overset{O}{\overset{\|}{C}}-$ group), 8.65 (br s, chain methylene protons). Thus the spectral data established the structure as methyl 10-chloro-11-oximino-undecanoate (XIV). Further support to the structure was obtained from the analysis of the corresponding carbonyl compound (XVI) obtained by the deoximation of the product (XIV) with the help of levulinic and hydrochloric acid[110]. The deoximation formed a compound (XVI) which was shown to have an aldehydic group. The presence of aldehydic group in product (XVI) was confirmed with the help of chemical tests and spectroscopy. It gave yellow colour on heating with NaOH and reduces Fehling's solution. It also gave a positive DNP test on TLC. IR spectrum showed the disappearance of bands at 3300 and 1690 cm^{-1} (shown by oxime) and new bands appeared at 1710 (C=O) and 2800 (aldehyde C-H str) cm^{-1} attributed to the aldehydic function.

Characterisation of compound (XV)

The compound (XV), which migrated ahead of oxime (XIV) on TLC plate, analysed for $C_{12}H_{21}N_2O_4Cl$ (positive Beilstein test). The IR spectrum showed bands at 1630 (C=N) and 1550 (NO_2) cm^{-1} characteristic of nitrimino group. NMR showed significant signals at τ 3.94 for one proton at C-11 (-C<u>H</u>=N.NO_2) and a multiplet centered at τ 5.4 for methine proton adjacent to chlorine atom (-C<u>H</u>Cl-). Other NMR signals usually displayed by the fatty acid esters (τ 6.34, 7.76, and 8.68) were also present. The product was thus assigned the structure as methyl 10-chloro-11-nitriminoundecanoate.

The formation of only one isomer (XII) in the nitroso-chlorination of 10-undecenoate indicated that the reaction is regiospecific and addition of NOCl is in accordance with the Markownikoff's rule. The exclusive formation of(XII) as primary product in the NOCl addition to methyl 10-undecenoate is consistent with the intermediacy of a three membered ring ion, A, opening of which proceeds <u>via</u> the lower energy transition state (B rather than D when R can stabilise an incipient positive charge).

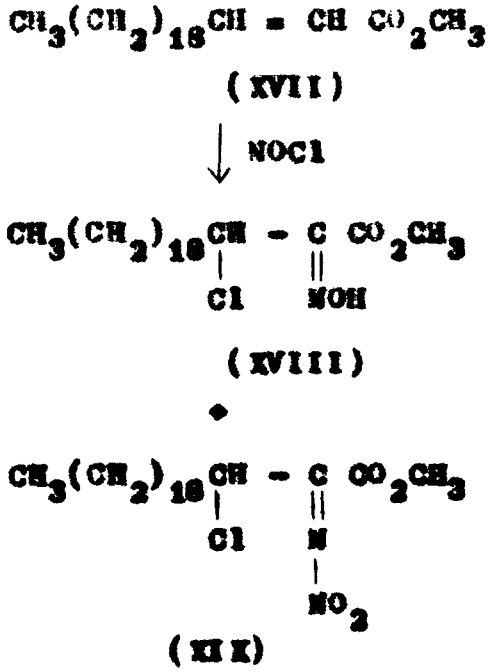


Further the results showed that nitrosochlorination is the only primary reaction and the secondary products were formed as a result of two simultaneous pathways. Dimerization leads to product (XIII) and isomerization followed by oxidation yields an oxime (XII) and nitrimine (IX). Dimerization seems to be much more feasible in methyl 10-undecenoate than in methyl oleate probably due to steric reasons. Isomerization of nitroso compound to an oxime also seems to be faster than in the case of methyl oleate as evidenced by the yields. Chloronitrimine formation was found to be ~ 8% in yield when excess of nitrosyl chloride was used. None of the remainder is oxidized to the chloronitro compound apparently because of isomerization to chloroxime and subsequent oxidation to chloronitrimine.

Nitrosochlorination of methyl docos-trans-2-enoate (XVII)

Methylene chloride solution of methyl docos-trans-2-enoate was treated with NOCl (in situ) in a stoppered flask at 0-5° by keeping in a refrigerator for about a month. Monitoring the reaction by TLC showed that the reaction is extremely slow and that sample from the reaction mixture after the work-up revealed the presence of three components, which were subjected to column chromatographic separation. The major component was found to be the starting material. Only about 10% of the compound (XVII) has reacted. The products were characterized on the basis of elemental analysis, IR and NMR.

Scheme 3



Characterisation of the compound (XVIII)

The compound (XVIII) gave satisfactory elemental analysis for $C_{22}H_{44}O_3NCl$ (positive Beilstein test). Compound (XVIII) gave informative IR spectrum with bands at 3300 and 1640 cm^{-1} indicative of the oximino group. NMR spectroscopy was useful in confirming the structure of compound (XVIII) as methyl 2-oximino-3-chlorodocosanoate. A signal was obtained for a single proton at τ 2.76 (signal disappeared on addition of D_2O) attributed to the oximino group proton (=NO<u>H</u>). A triplet was observed at τ 6.1 for methine proton adjacent to chlorine atom. The chemical shift and multiplicity of -C<u>H</u>Cl- signal confirms the attachment of chlorine atom to C-3 instead of C-2. Other proton signals were exhibited at τ 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OC\underline{H}_3$), 8.75 (br s, chain methylene protons) and 9.12 (distorted t, 3H, terminal methyl group).

Characterisation of the compound (XIX)

The compound (XIX) was analysed for $C_{23}H_{43}O_4N_2Cl$. It responded to Beilstein test. Aside from elemental analysis, proof of structure for compound (XIX) was also obtained from spectroscopic evidence. The IR spectrum gave bands at 1640(C=N), 1550 and 1360 (NO_2) cm^{-1} characteristic of nitrimino group. The NMR data were also consistent with the structure methyl

2-nitrimino-3-chlorodocosonoate for the compound (XIX). It exhibited a triplet at τ 5.9 for methine proton adjacent to chlorine atom (-CHCl-). Usual fatty ester signals were also observed at τ 6.36 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.65 (br s, chain methylene protons) and 9.1 (distorted t, 3H, terminal methyl). The chemical shift and multiplicity of methine proton signal adjacent to chlorine atom confirm the attachment of chlorine atom to carbon-3 as in the case of oximino compound (XVIII).

The formation of compounds (XVIII) and (XIX) can well be explained through the nitrosochlorination of compound (XVII) as the primary reaction. The isomerisation of nitroso compound will give an oxime (XVIII) which on subsequent oxidation by NOCl will provide a nitrimine (XIX).

$$CH_3(CH_2)CH{=}CH\ CO_2CH_3 \xrightarrow{NOCl} CH_3(CH_2)\underset{Cl}{CH} - \underset{NO}{CH}\ CO_2CH_3$$

(XVII)

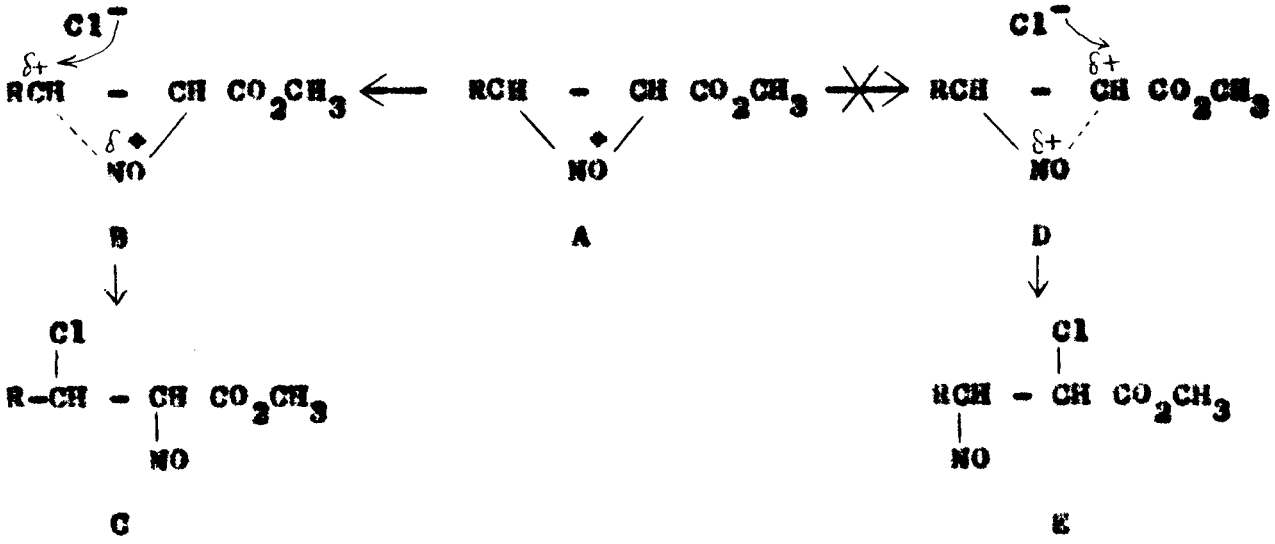
$$\downarrow \text{Isomerization}$$

$$CH_3(CH_2)\underset{Cl}{CH} - \underset{NOH}{C}\ CO_2CH_3 \xrightarrow{NOCl} CH_3(CH_2)\underset{Cl}{CH} - \underset{\underset{NO_2}{|}}{\overset{\overset{N}{\|}}{C}}\ CO_2CH_3$$

(XVIII) (XIX)

In case of α,β-unsaturated acid (XVII) only one isomer resulted during nitrosochlorination. The presence of electron-

withdrawing group ($-\overset{O}{\overset{\|}{C}}-OCH_3$) adjacent to double band is involved in opening of nitrosonium ion intermediates. The electron withdrawing group will destablize the transition state D relative to B and hence NO-carbonyl regiospecific NOCl adduct C will be formed.



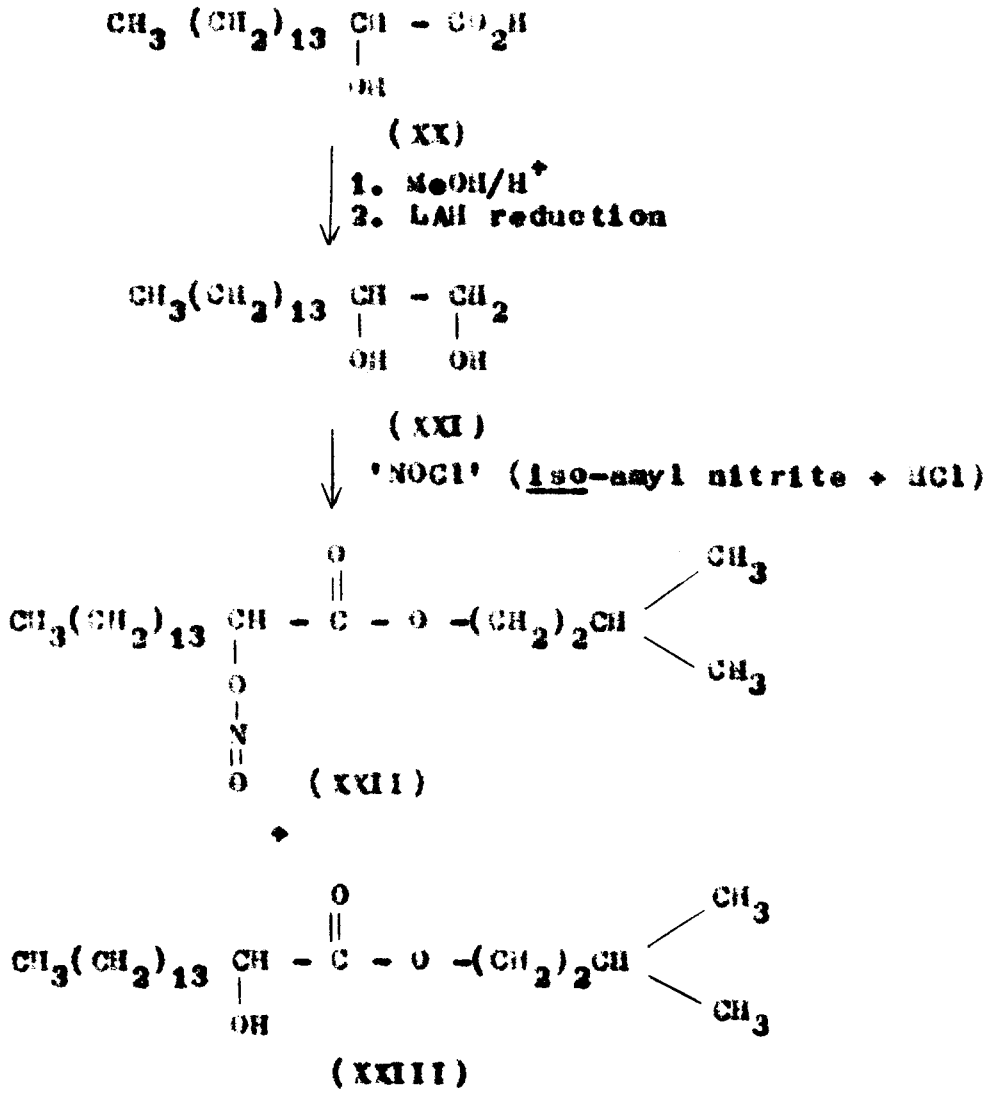
The considerable slow rate of the reaction is attributed to the proximity of the double bond to the electron withdrawing ester carbonyl function. Thus the decrease in the nucleophilic character of α,β-unsaturation slows down the electrophilic reaction of NOCl addition.

Reaction of nitrosyl chloride with fatty 1,2 diol (1,2-hexadecandiol, XXI)

The reaction of 1,2-diols with nitrosyl chloride has received little attention. The present work was undertaken in order to extend the investigation of NOCl reaction to fatty 1,2-diols, with a view to ascertain the nature of the reaction products and their spectral behaviour.

The diol used as a substrate was prepared from 2-hydroxyhexadecanoic acid (XX) which was obtained as one of the co-products during the preparation of C_{16} α,β -unsaturated acid. Methylene chloride solution of 1,2-hexadecandiol (XXI), on treatment with an excess of nitrosyl chloride *in situ* at room temperature afforded a mixture of two products together with some unreacted compound as evidenced by analytical TLC. The components were resolved by silica column. Structures of compounds (XXII and XXIII) were corroborated by micro-analysis, IR, NMR and Mass. The reaction carried out in the present investigation is outlined in Scheme 4.

Scheme 4



Characterisation of compound (XXII)

Microanalysis of the compound (XXII) supported the formula $C_{21}H_{41}NO_4$ (negative Beilstein test). IR spectrum exhibited band at 1630 cm^{-1} indicative of a nitrito group

(C-O-N=O). IR band at 1730 cm^{-1} was also present showing the presence of an ester carbonyl group. NMR spectrum showed a two proton resonance at τ 5.9 (m) corresponding to methylene group adjacent to oxygen atom (-O-$\underline{CH}_2$-) and a signal at τ 8.4 was exhibited for methine proton (-$\underline{CH}<$). A broad singlet at τ8.7 was observed for shielded methylene protons. NMR spectrum also showed an apparent doublet centered at τ 9.1 corresponding to three methyls. The structure of compound (XXII), as <u>iso</u>-amyl 2-nitritohexadecanoate was further supported by mass spectrometry (Fig. 7, Sheet VII). The genesis of important fragment ions are discussed.

The mass spectrum of compound (XXII) gave no molecular ion peak at m/e 371 ($C_{21}H_{41}NO_4$). The highest peak was observed at m/e 201 with other important peaks at m/e 191, 188, 187, 173, 137, 117, 87, 86, 85, 73, 72, 71 (base peak), 70, 69, 58, 57, 56 and 55 and other low mass ion species. The formation of some of the more significant ions can be rationalised according to Schemes below. These fragmentation pathways are tentative since the mass spectra of appropriate deuterated analogs have not been examined.

m/e 201 $[M^{\cdot+}(371)-CH_3-155]$

This fragment ion which agrees with the loss of $-CH_3$ and loss of mass unit 155 from the expected molecular ion is shown in Scheme 5.

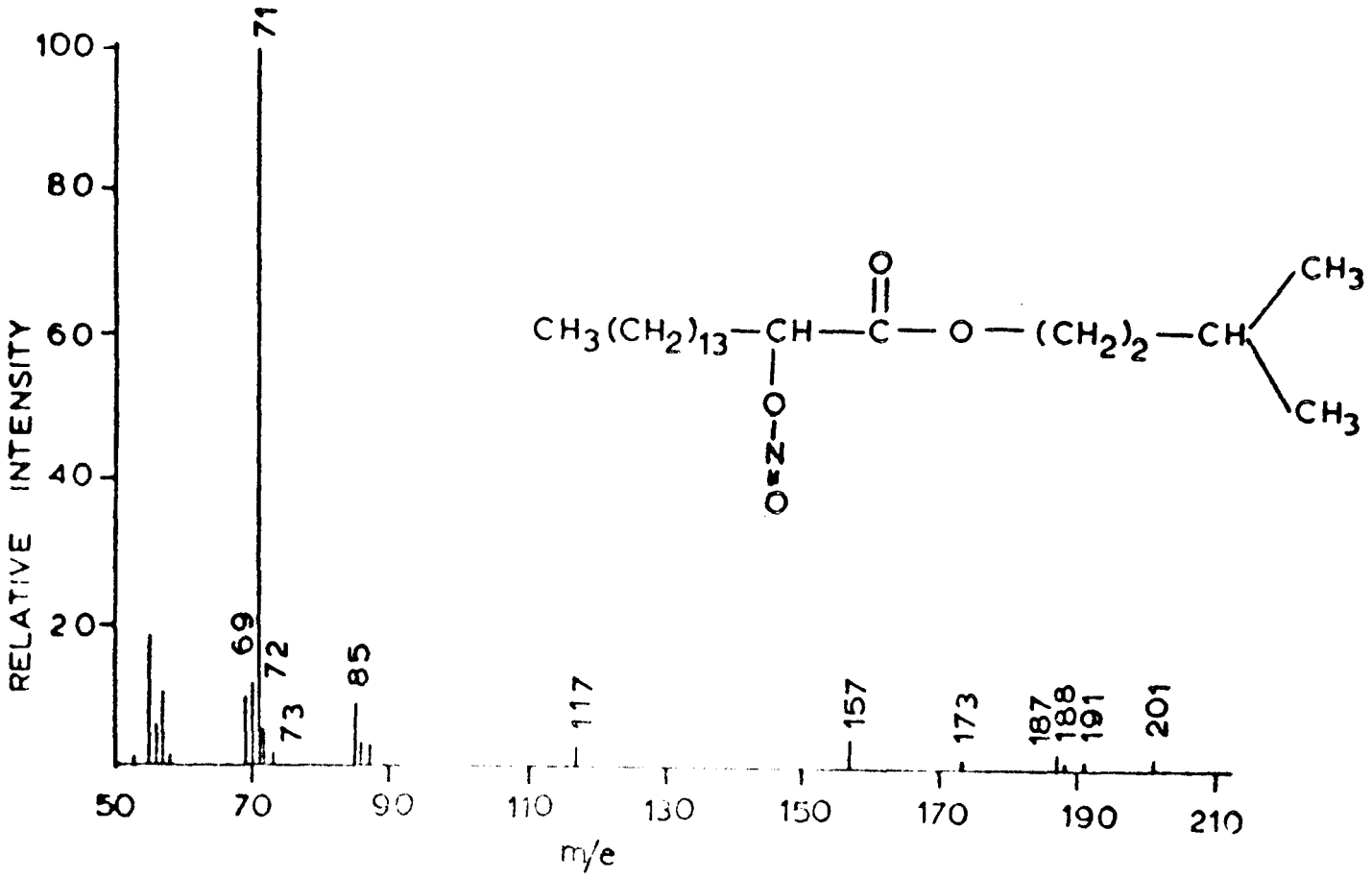
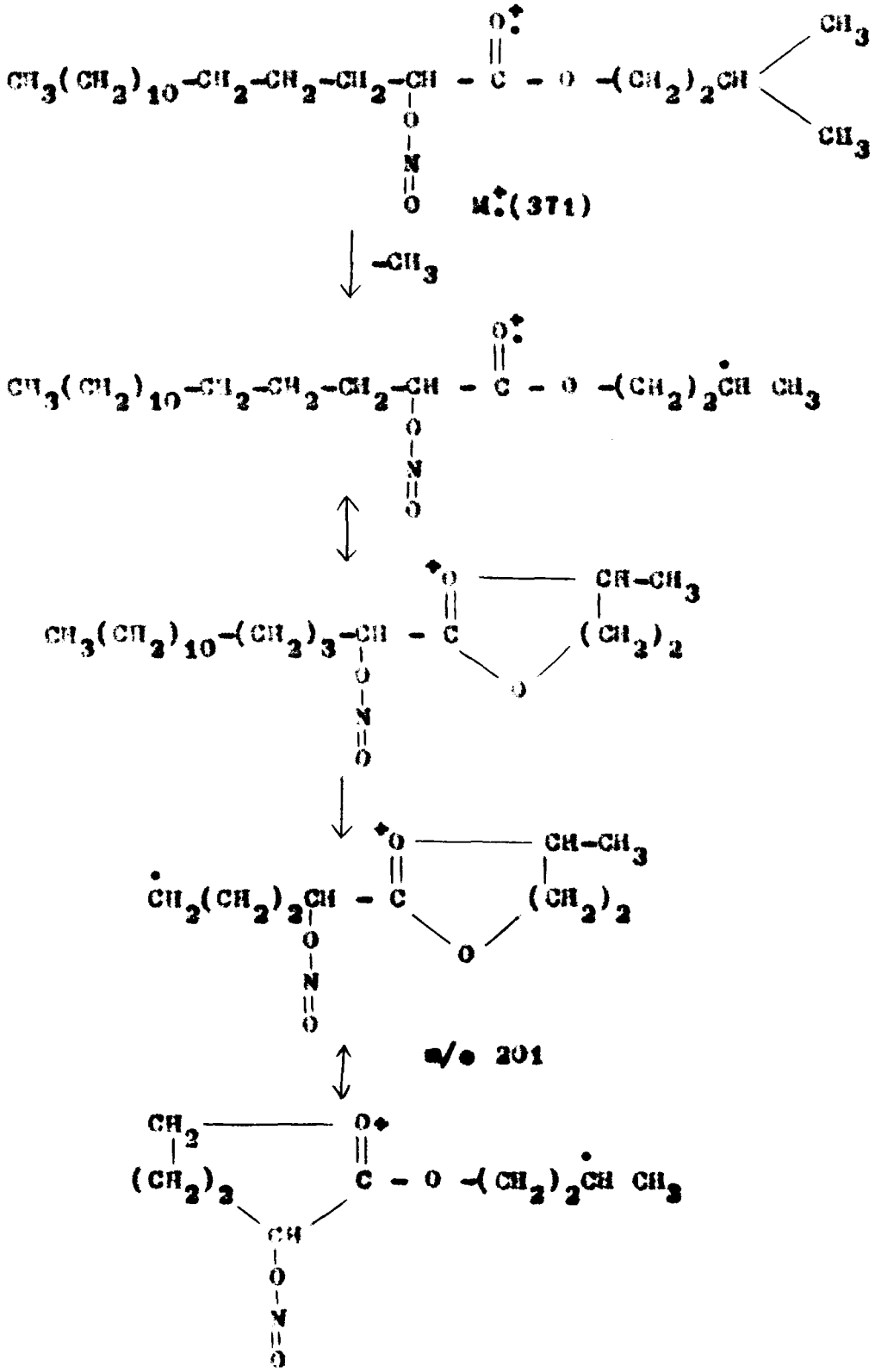


Fig.7. Mass spectrum of <u>iso</u>-amyl 2-nitritohexadecanoate (XVIII)

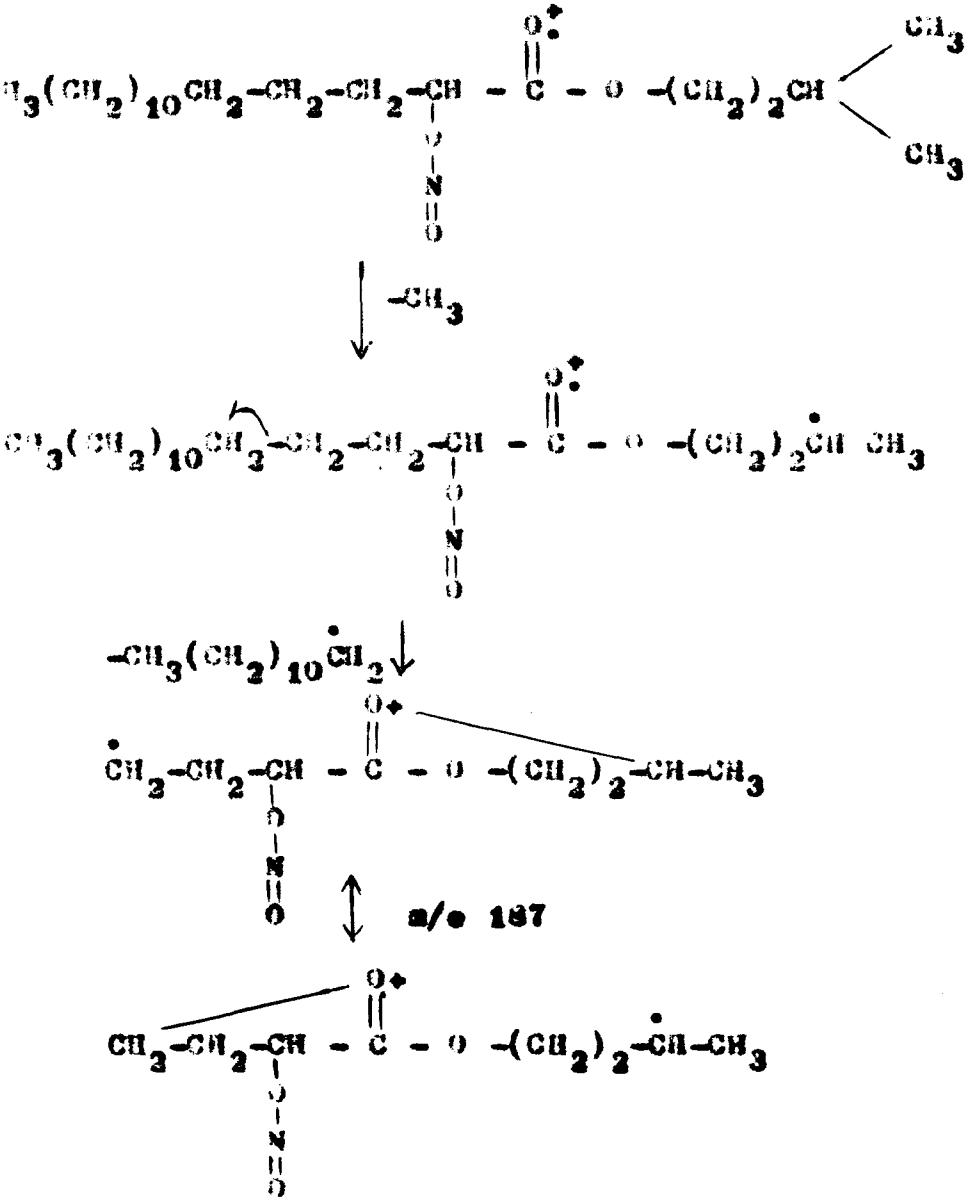
Scheme 3



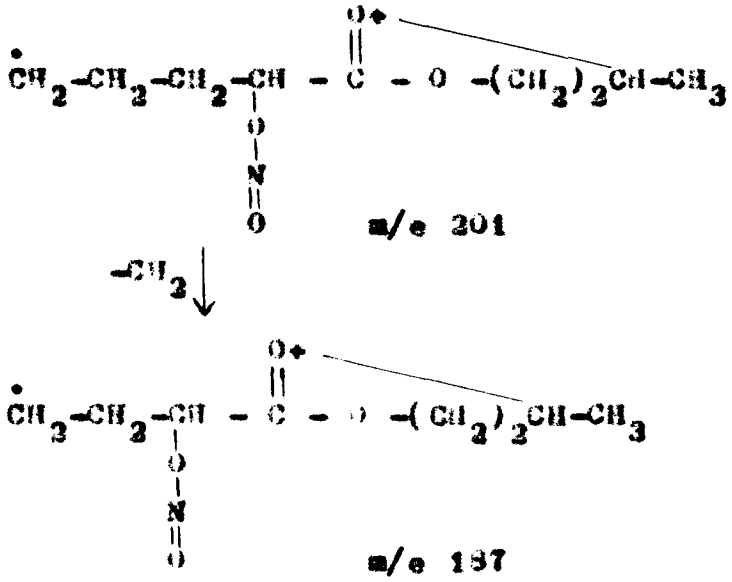
m/e 187 ($C_9H_{13}O_4N$)($M^{\bullet+}$ - 184) or (m/e 201-14)

Obviously, this fragment ion is obtained either (a) by the loss of mass units 15 and 169 from the molecular ion or (b) by the loss of mass unit 14 from m/e 201 (Scheme 6).

Scheme 6



b. From Scheme 5 (201-14)



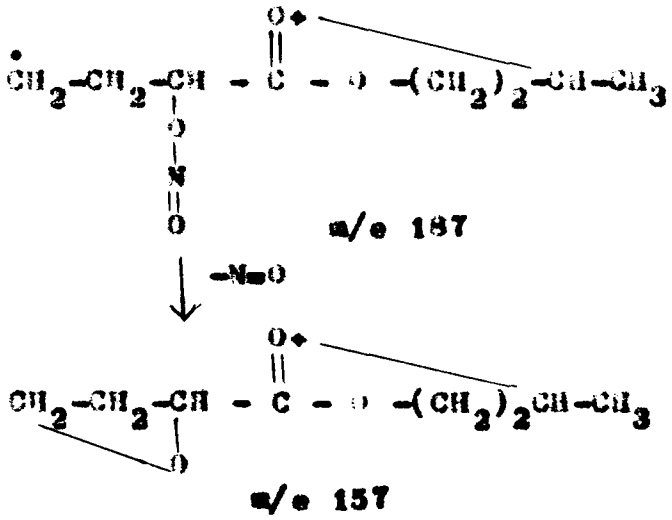
m/e 201

m/e 187

m/e 157 (m/e 187-N=O)

The fragment ion m/e 157 can be conveniently shown as arising from the ion m/e 187 (Scheme 7).

Scheme 7



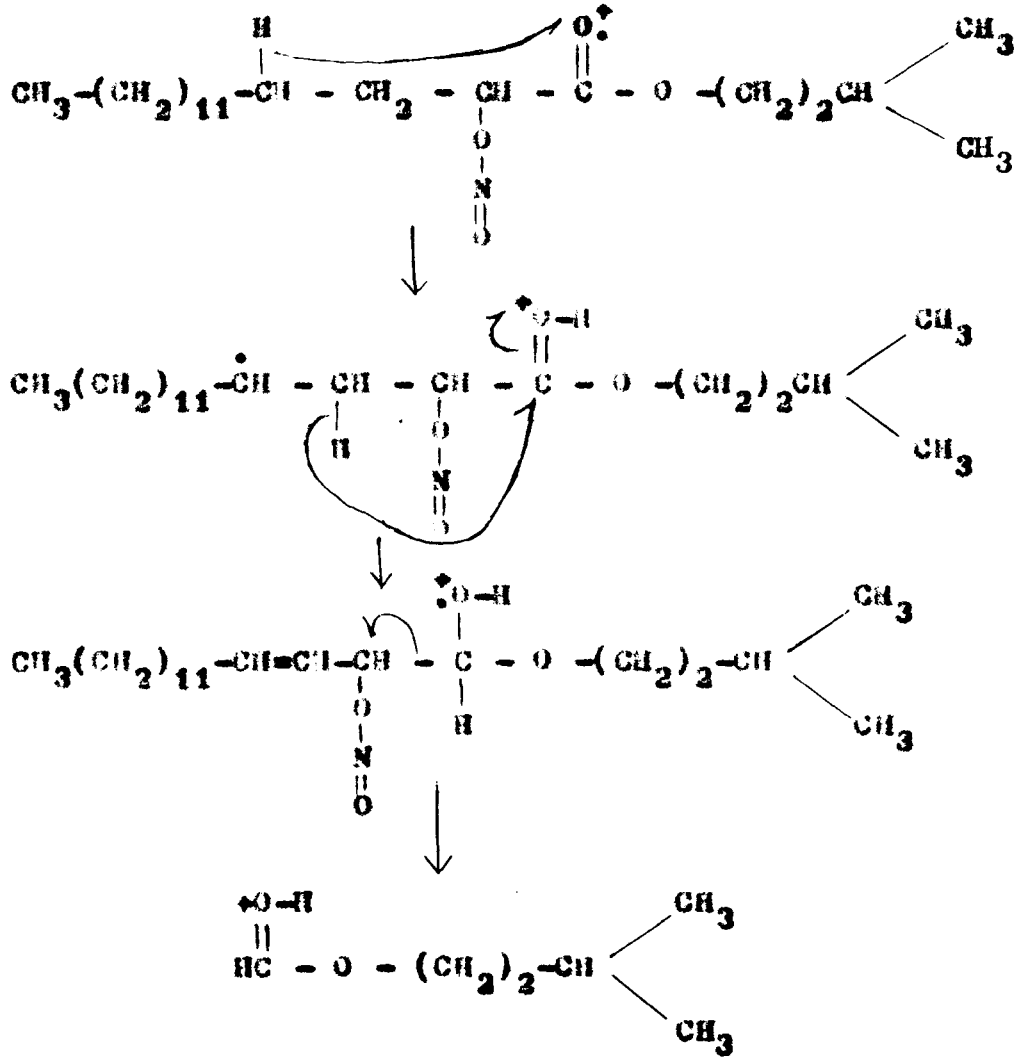
m/e 187

m/e 157

m/e 117

The formation of this ion may be rationalized according to Scheme given below:

Scheme 8

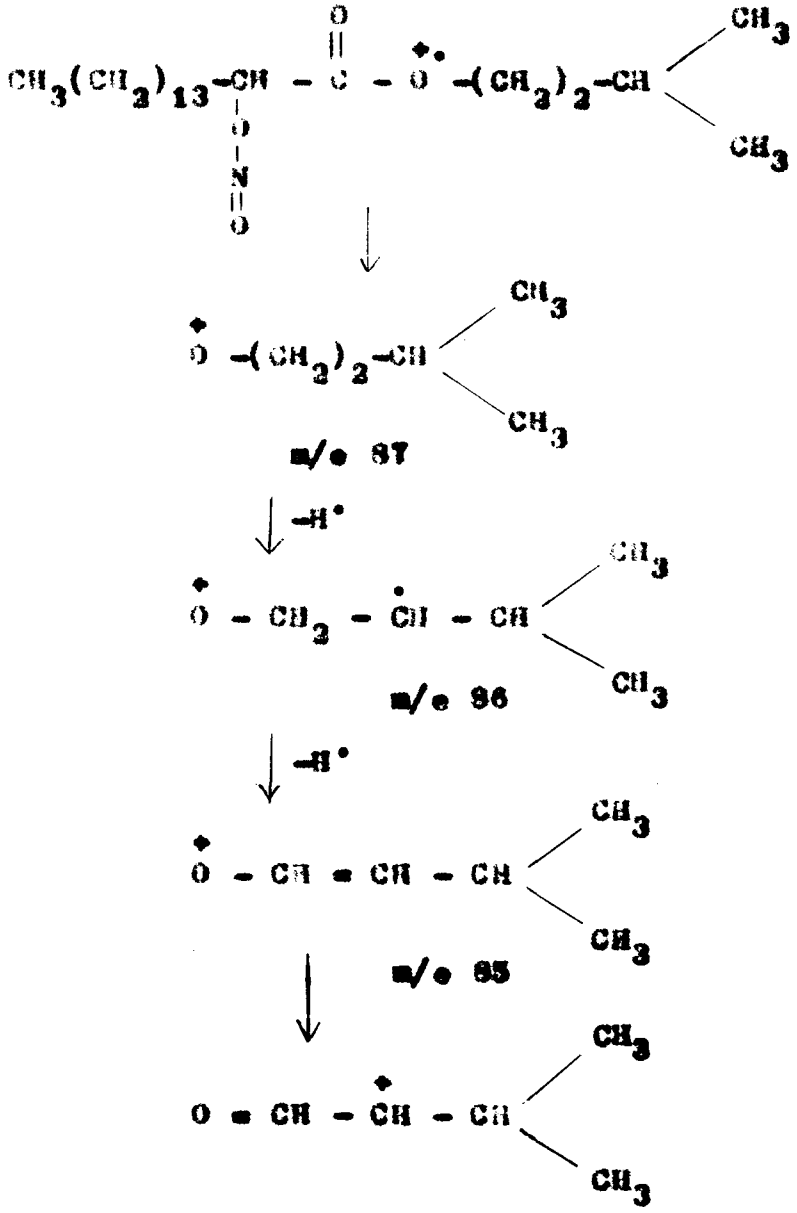


m/e 117

m/e 97, 96 and 95

The mass ion peaks at m/e 97, 96, and 95 may be explained to arise as below:

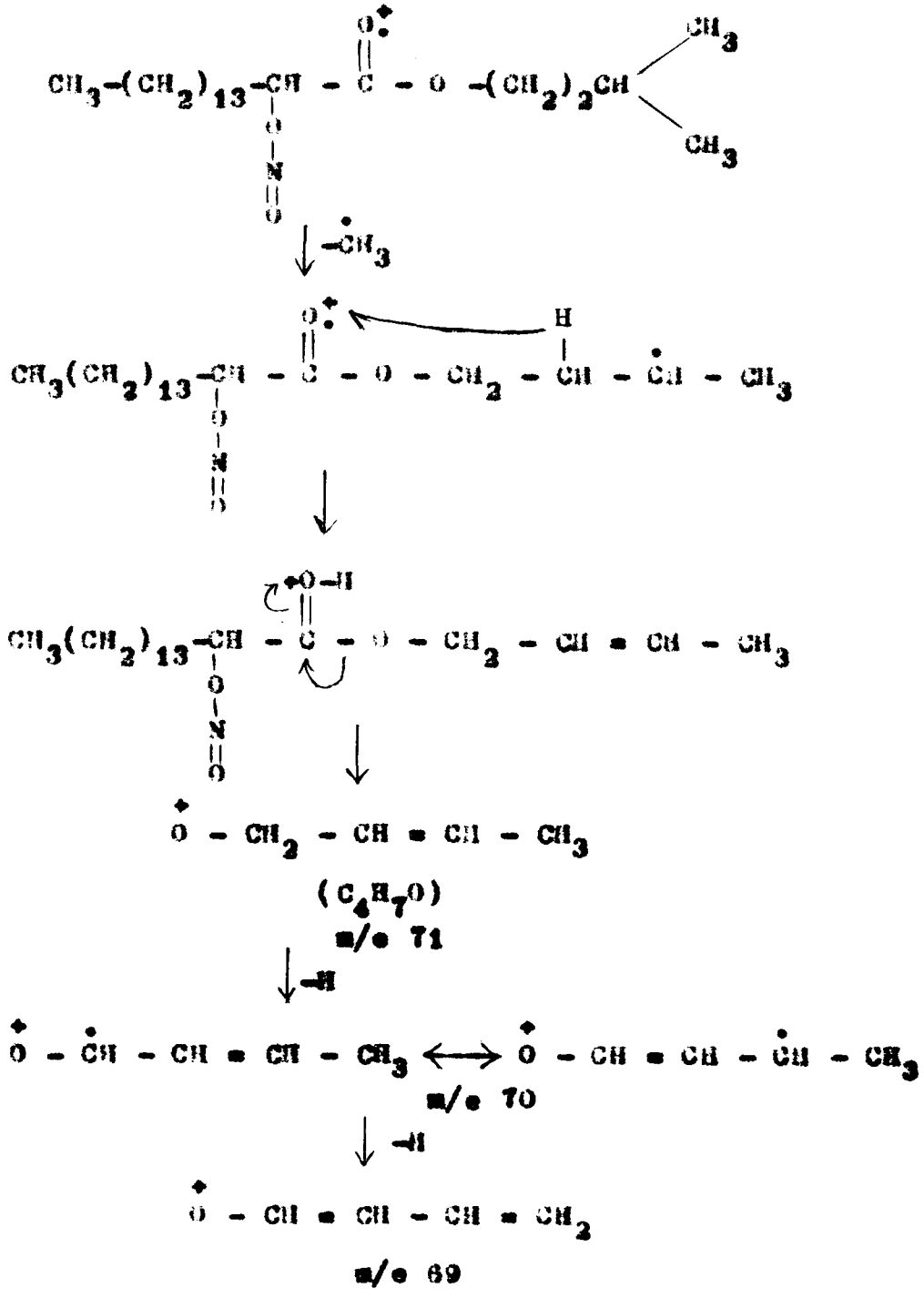
Scheme 9



m/e 71 (base peak), 70 and 69

The fragment ion peak at m/e 71 constitute the base peak of the spectrum (Scheme 10).

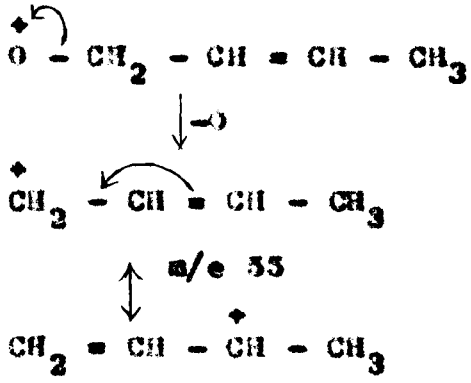
Scheme 10



m/e 55

This hydrocarbon fragment ion may be shown to arise from the ion m/e 71 as below.

Scheme 11



Characterization of compound (XXIII)

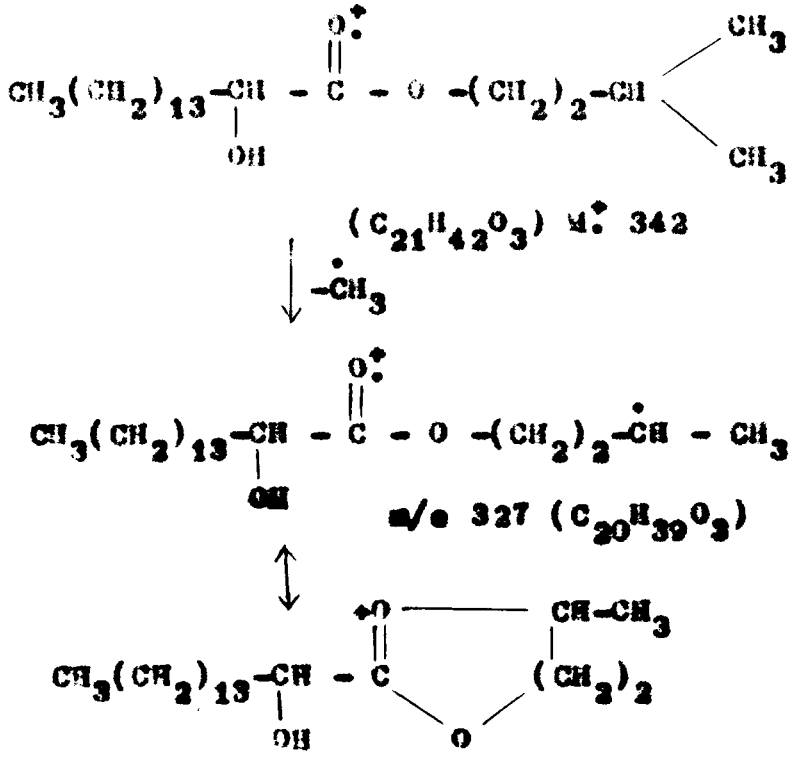
The compound (XXIII) was analyzed correctly for $C_{21}H_{42}O_3$ (negative Beilstein test). IR spectrum displayed bands at 3350 cm^{-1} characteristic of hydroxyl group and at 1730 cm^{-1} due to ester carbonyl group. The NMR spectrum showed an apparent multiplet centered at τ 5.9 due to methylene protons adjacent to oxygen atom ($-O-\underline{CH_2}-$), a signal at τ 9.4 due to methine proton ($-\underline{CH}(CH_3)_2$) and an apparent doublet centered at τ 9.05 due to methyl protons (9H). A broad singlet at τ 8.65 for shielded methylenes was also observed. The NMR spectrum agrees well with the assigned structure for compound (XXIII) as iso-amyl 2-hydroxyhexadecanoate.

The mass spectrum of iso-amyl 2-hydroxyhexadecanoate (XXIII) (Fig.8,Sheet VIII) also gave no molecular peak at m/e 342 ($C_{21}H_{42}O_3$), but other significant peaks at m/e 327 ($M-CH_3$), 313, 295, 294, 286, 284, 225, 174, 133, 132 (base peak), 116, 113, 111, 109, 104, 100, 99, 97, 95, 90, 85, 84, 83, 82, 81, 74, 71, 70, 69, 68, 67, 57, 56, 55, and 54.

m/e 327 ($M-CH_3$)

The following mechanism has been proposed to account for the loss of CH_3 from the molecular ion m/e 342 (Scheme 12).

Scheme 12



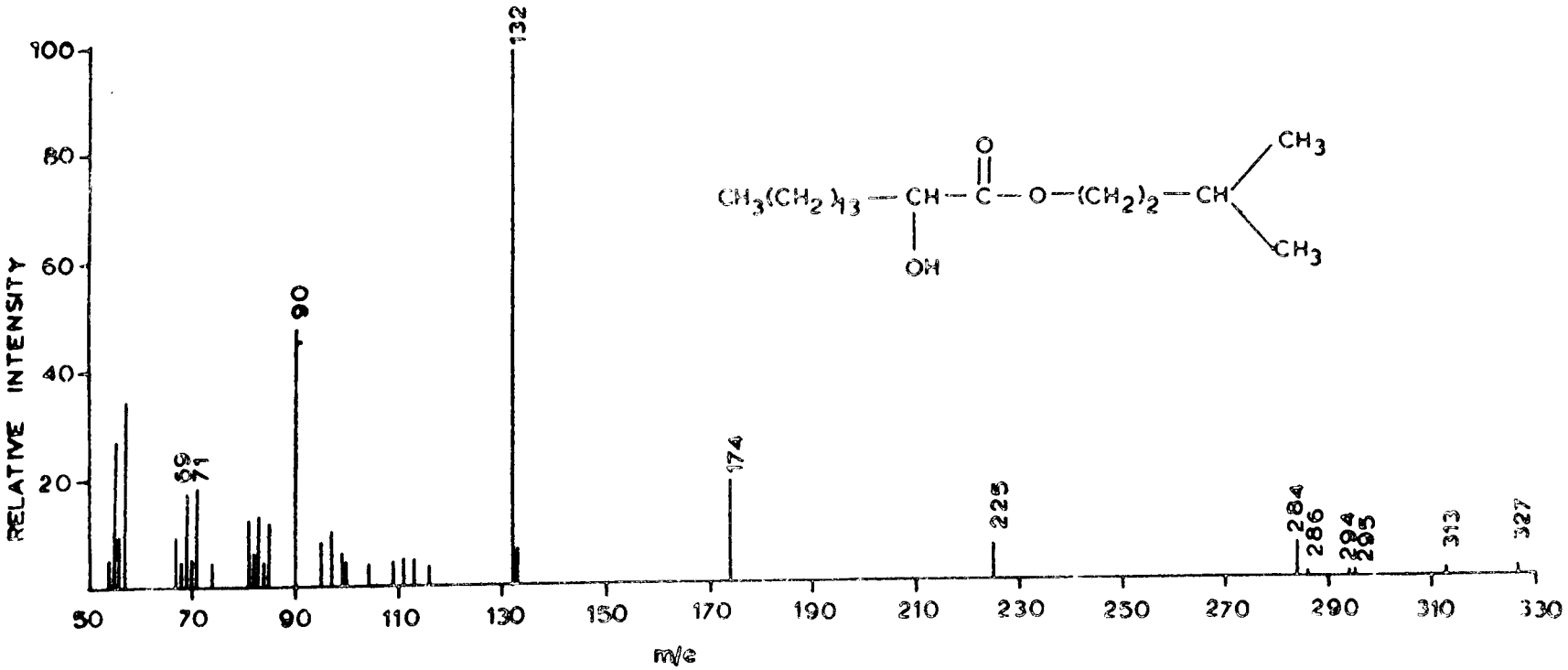
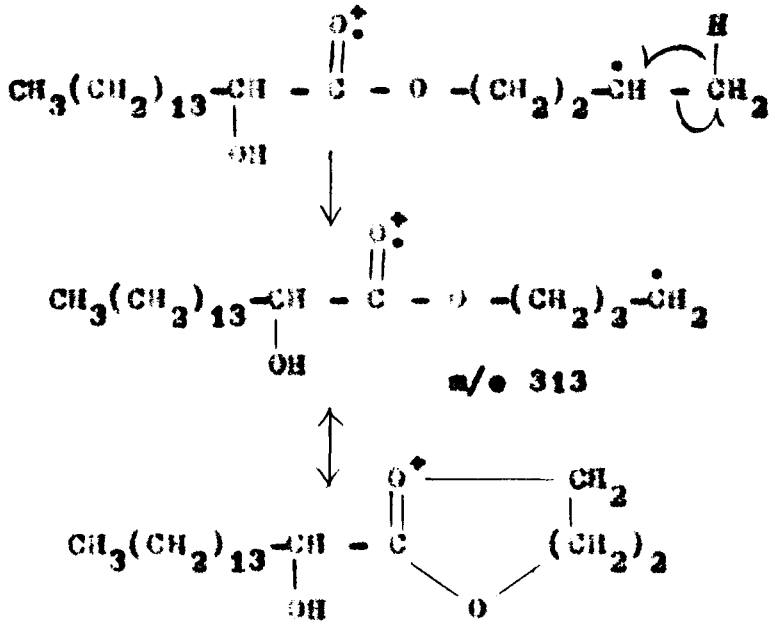


Fig.9. Mass spectrum of iso-amyl 3-hydroxyhexadecanoate (XIX)

m/e 313 (m/e 327-14)

The fragment ion m/e 313 can be rationally derived from the ion m/e 327, as depicted in Scheme 13.

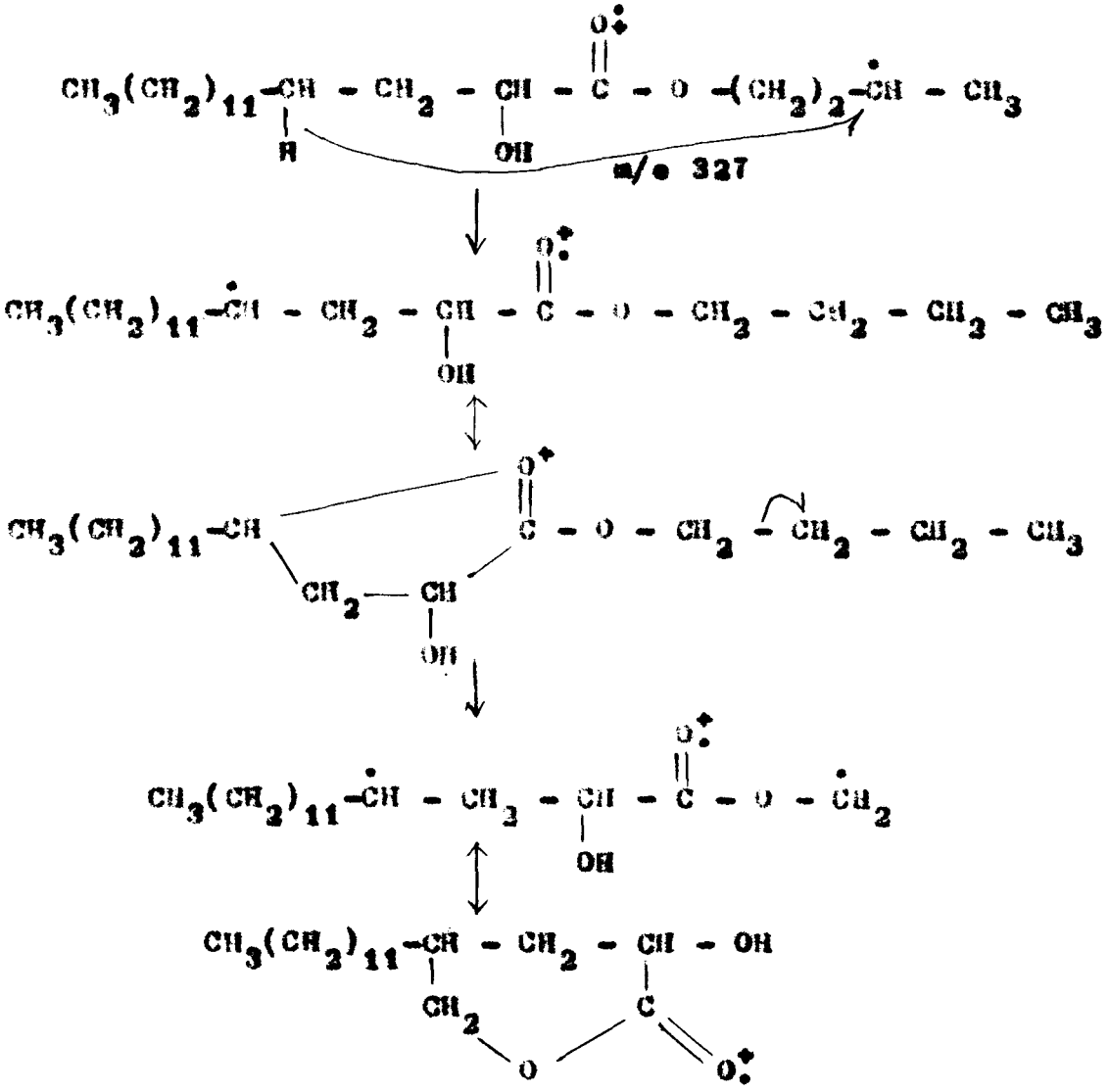
Scheme 13



m/e 284

The fragment ion m/e 284 may result by the loss of mass unit 43 from m/e 327 (Scheme 14).

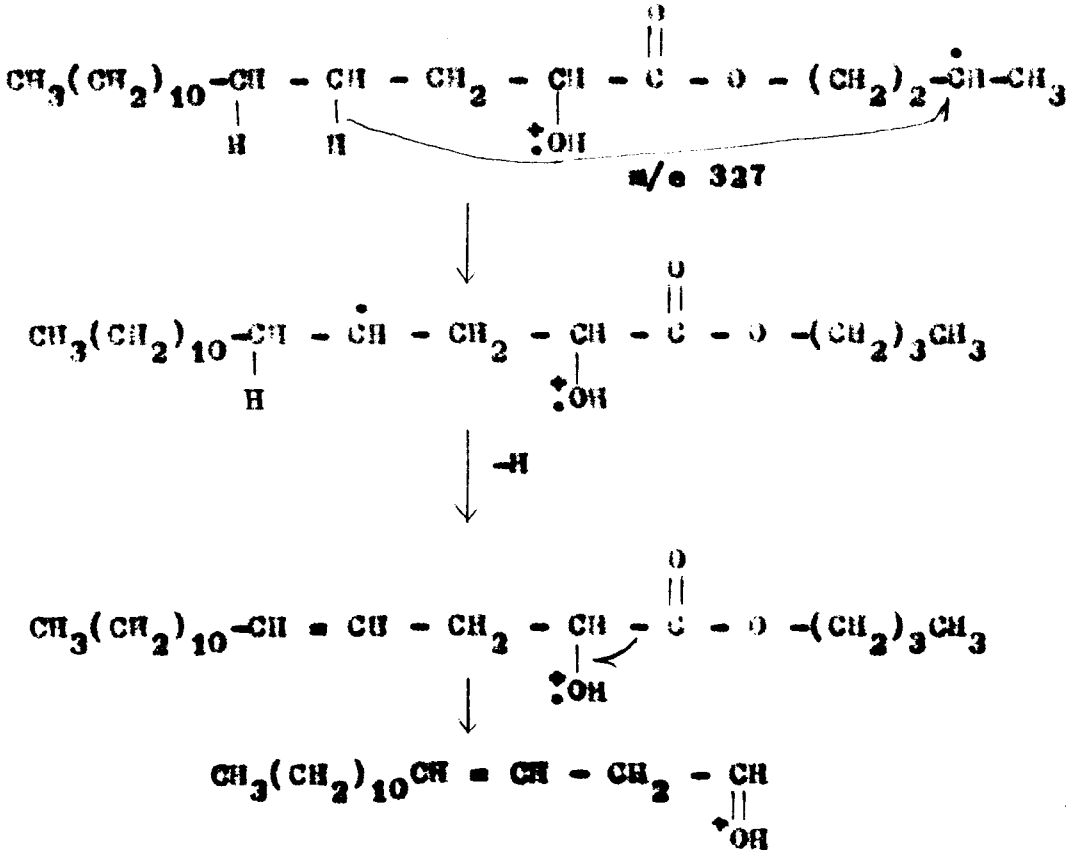
Scheme 14



m/e 225

The fragment ion m/e 225 is diagnostic in nature and it fixes the structure of compound (XXIII) as iso-amyl 2-hydroxy-hexadecanoate. The ion formation may be attributed to the cleavage between carbon 1 and 3 (Scheme 15).

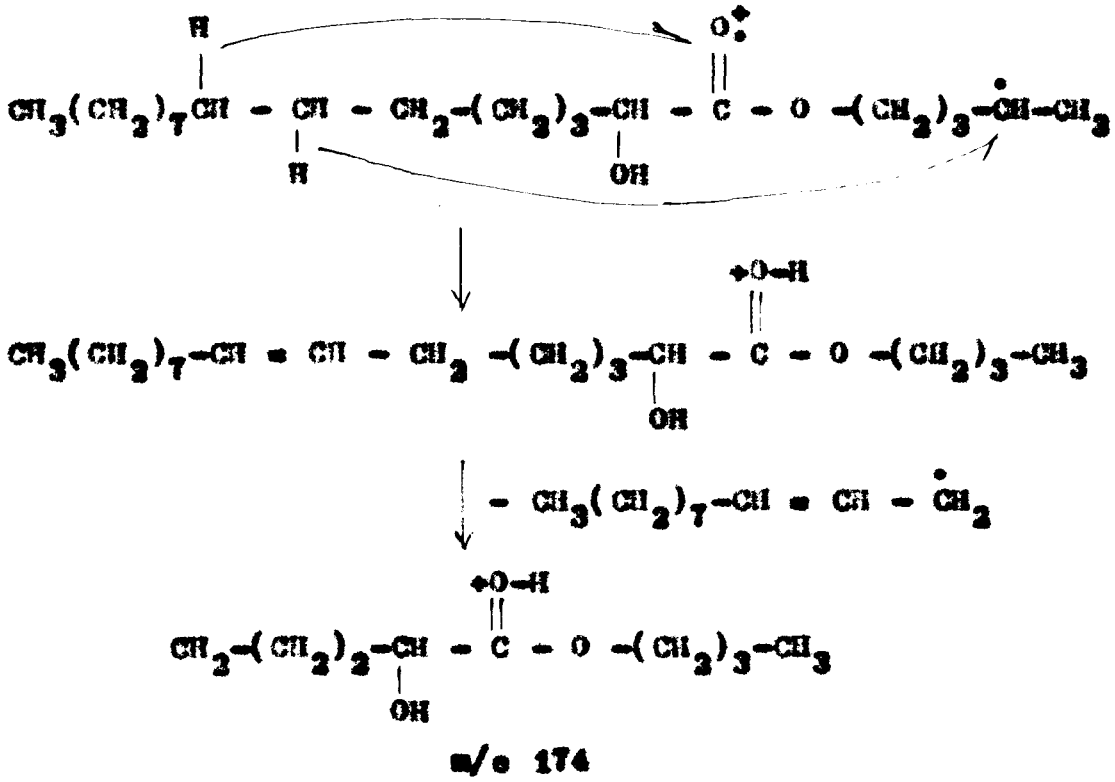
Scheme 15



m/e 174

The fragment ion peak at m/e 174 is fairly strong and this perhaps results by the loss of $CH_3(CH_2)_7CH{=}CH\dot{C}H_2$ from the ion m/e 327 (Scheme 16).

Scheme 16

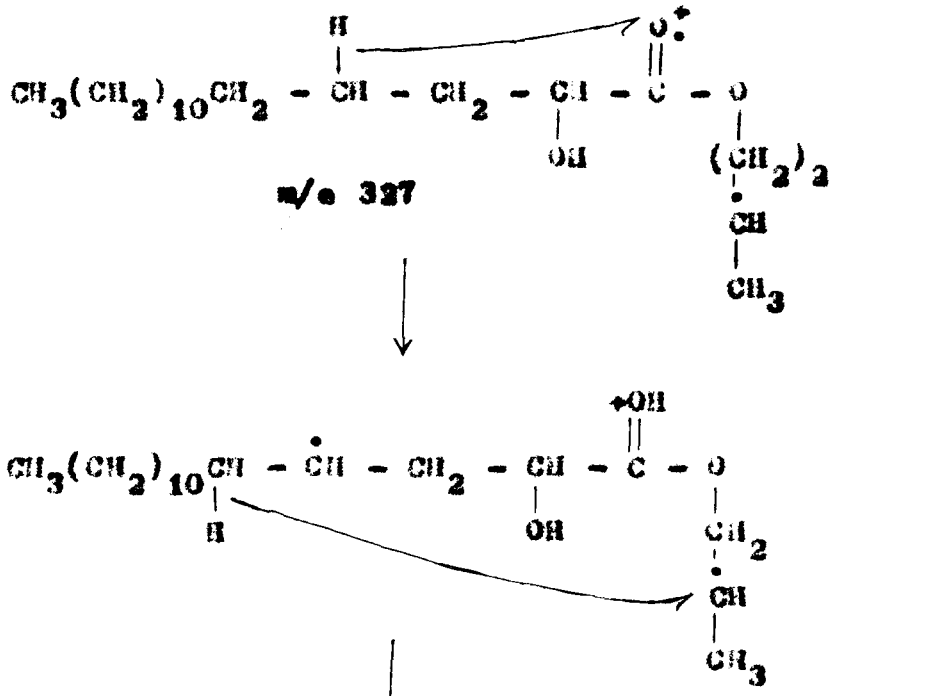


m/e 132

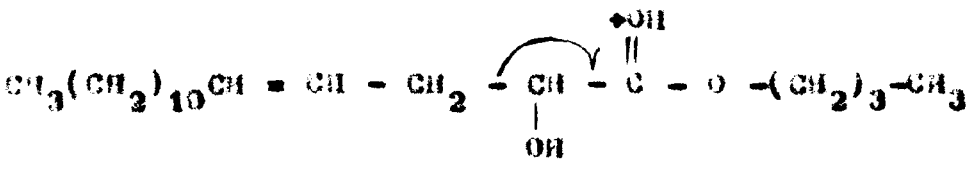
The fragment ion peak at m/e 132 constitutes the base peak of the spectrum which indicates the position of the substituent -OH at C 3 atom of the chain. This fragment ion can be shown to arise by the migration of two hydrogens and simultaneous cleavage of 2,3 carbon-carbon single bond from m/e 327 (Scheme 17).

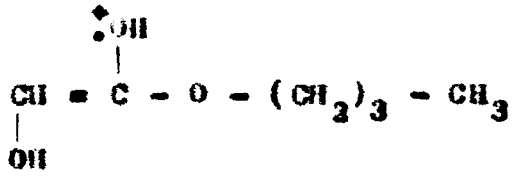
Scheme 17

a.



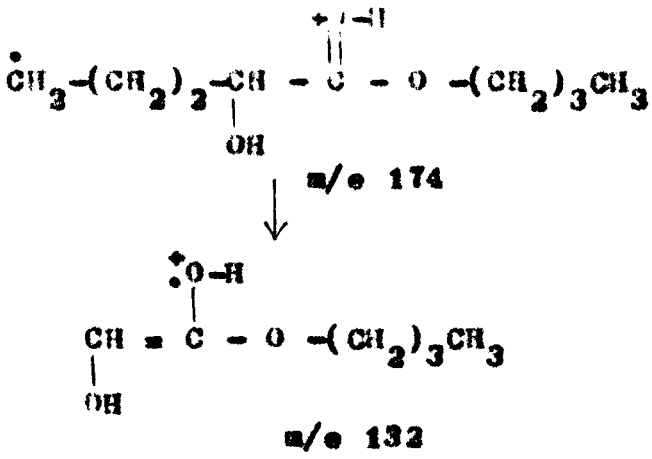
m/e 327





m/e 132

b.



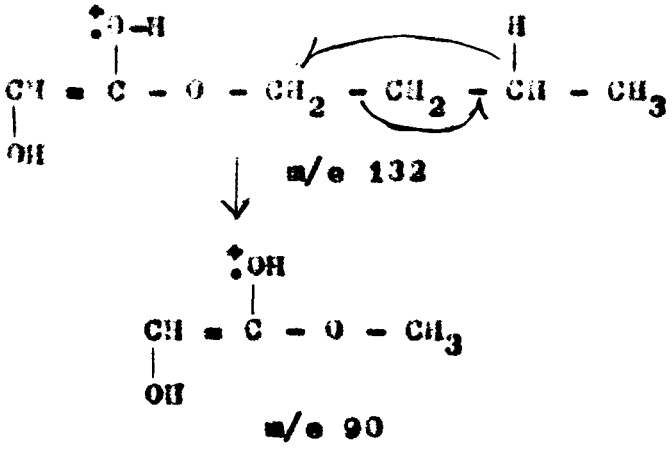
m/e 174

m/e 132

m/e 90

The fragment ion m/e 90 may result by the loss of mass unit 42 from m/e 132 (Scheme 18).

Scheme 18

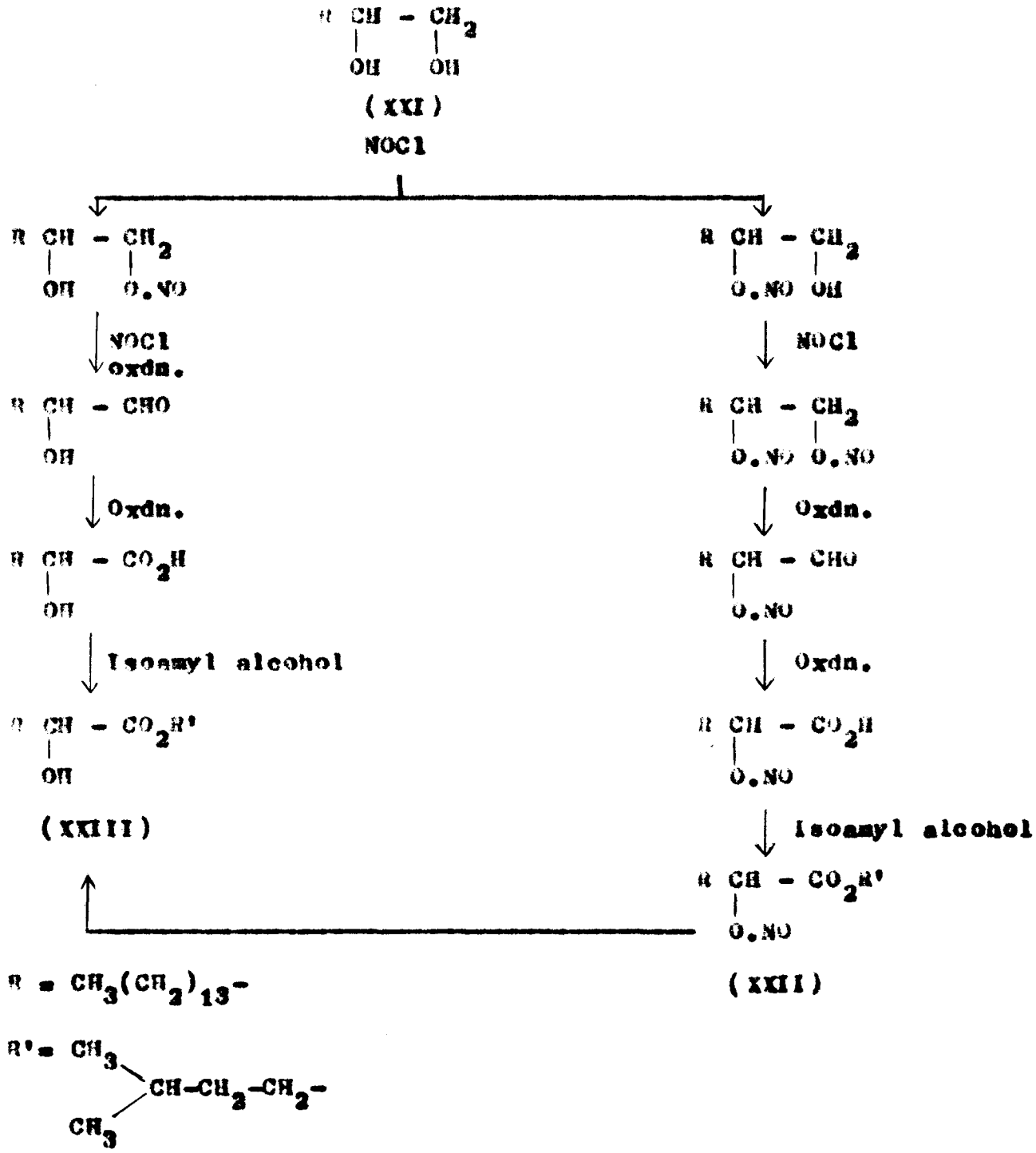


m/e 132

m/e 90

The probable route for the formation of compounds (XXII) and (XXIII) from (XXI) may be shown as under (Scheme 19).

Scheme 19


R CH - CH2
OH OH
(XXI)
NOCl
R CH - CH2
OH O.NO
NOCl oxdn.
R CH - CHO
OH
Oxdn.
R CH - CO2H
OH
Isoamyl alcohol
R CH - CO2R'
OH
(XXIII)
R CH - CH2
O.NO OH
NOCl
R CH - CH2
O.NO O.NO
Oxdn.
R CH - CHO
O.NO
Oxdn.
R CH - CO2H
O.NO
Isoamyl alcohol
R CH - CO2R'
O.NO
(XXII)
R = CH3(CH2)13-
R' = CH3 CH-CH2-CH2-
CH3

Reaction of nitrosyl chloride with 10,11-epoxyundecanoic acid (XXV)

It is known that the oxirane group is highly reactive and undergoes a wide variety of ring-opening reactions with a broad range of electrophiles and nucleophiles. During the past decades, in particular, new and interesting reactions of the oxirane group have been described that provide new routes to other heterocyclic ring systems and functional groups. In continuation of a research programme carried out in our laboratory on new fatty acid reactions, the present investigation was undertaken. The reactions of many reagents with the oxirane group had been studied till date but no work had been reported on the reaction of oxiranes with nitrosyl chloride. A terminal epoxide was further selected for the present study with a view to study the direction of ring opening.

Preparation of 10,11-epoxyundecanoic acid (XXV) from 10-undecenoic acid (XXIV)

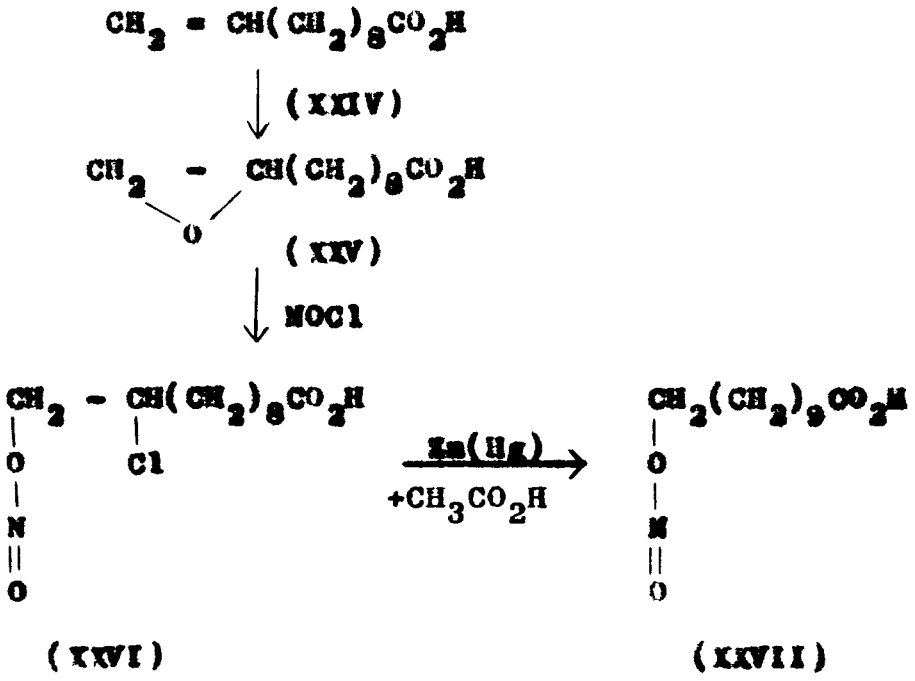
The 10-undecenoic acid (XXIV) was epoxidized to 10,11-epoxyundecanoic acid (XXV) by the procedure of Gunstone and Jacobsberg[111]. The epoxy acid (XXV) thus obtained was purified by using silica column.

Reaction of Compound (XXV) with NOCl

Nitrosyl chloride, needed for the reaction, was prepared by the action of sodium nitrite and HCl as described by Morton and Wilcox[112]. In this case the nitrosyl chloride was not generated in situ (iso-amyl alcohol + HCl) in order to avoid the contact of hydrochloric acid with the epoxy compound (XXV) as it will also react and open the epoxide ring.

Nitrosyl chloride gas was slowly passed through methylene chloride solution of 10,11-epoxyundecanoic acid at 0° with continuous stirring till whole of the compound has reacted as evidenced by TLC. Analytical TLC showed the quantitative yield of product (XXVI). A yellow oily liquid was obtained after the final work-up.

Scheme 20



(XXVI) (XXVII)

The product (XXVI) responded Beilstein test. The elemental analysis corresponded to the molecular formula $C_{11}H_{20}O_4NCl$. The IR spectrum showed a strong band at 1630 cm^{-1} displayed by the nitrites. The NMR spectrum (Fig. 9, Sheet IX) was decisive in arriving at a more firm conclusion regarding the structure of the compound as 10-chloro-11-nitritoundecanoic acid. NMR spectrum exhibited a doublet centred at τ 6.4 for two protons of methylene group adjacent to oxygen ($-O-\underline{CH}_2-$) showing the attachment of nitrito group at terminal carbon atom. The methine proton of chlorine-containing carbon displayed a signal at τ 5.9. Other usual NMR signals generally displayed by fatty acid chain were present at τ 2.47 ($-\overset{O}{\overset{\|}{C}}-O\underline{H}$, D_2O exchangeable), 7.7 (2H, protons α to carboxylic group), and 8.65 (br s, shielded methylene protons). In order to ascertain the respective positions occupied by nitrito and chloro groups the product (XXVI) was also subjected to reductive removal of chlorine atom yielding a product (XXVII) which gave negative Beilstein test. The NMR spectrum of compound (XXVII) showed the disappearance of signal at τ 5.9 showing thereby that the signal at τ 5.9 in compound (XXVI) was due to the methine proton adjacent to chlorine atom. These data supported the structure of compound (XXVI) as 10-chloro-11-nitritoundecanoic acid.

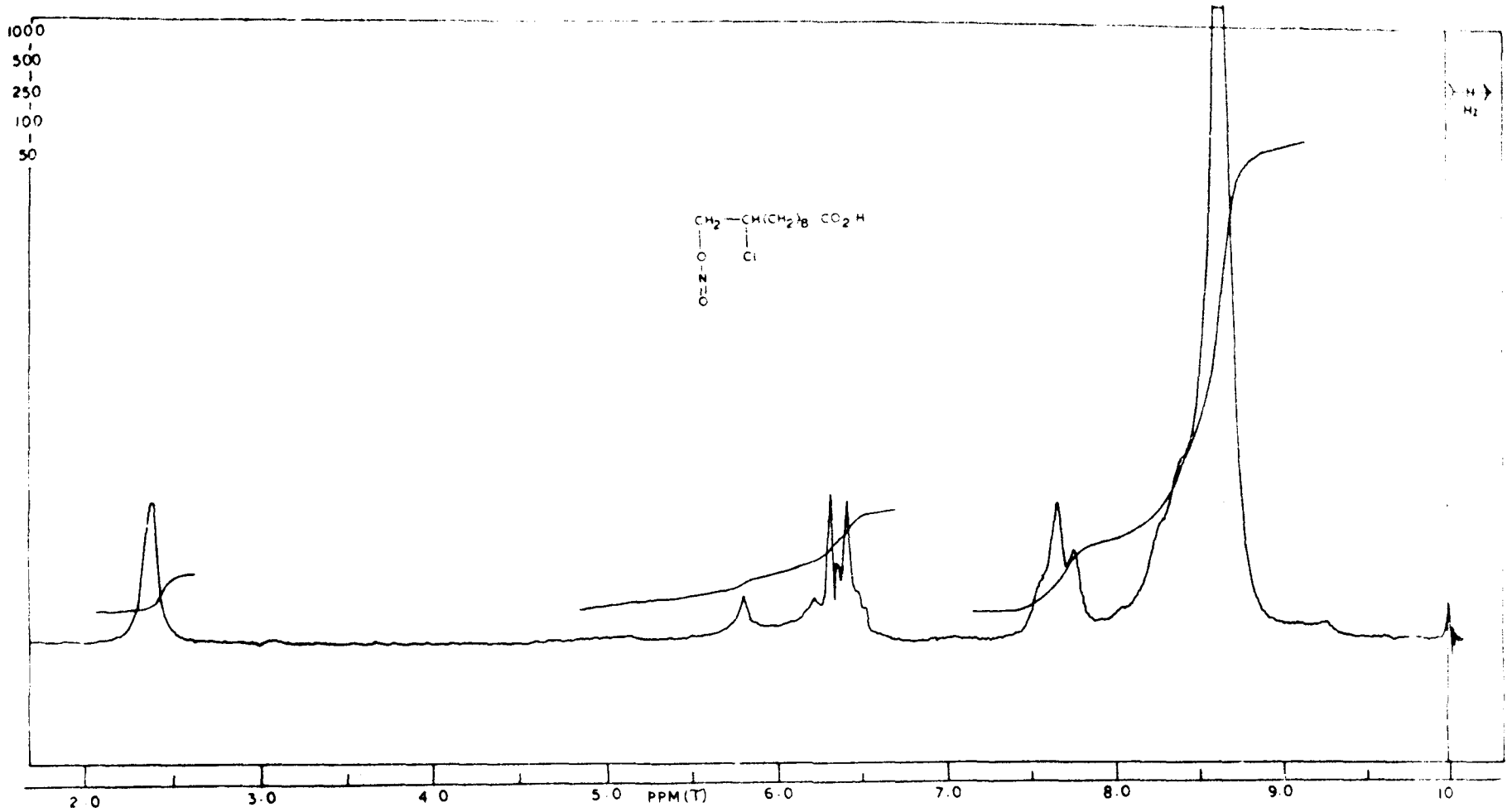
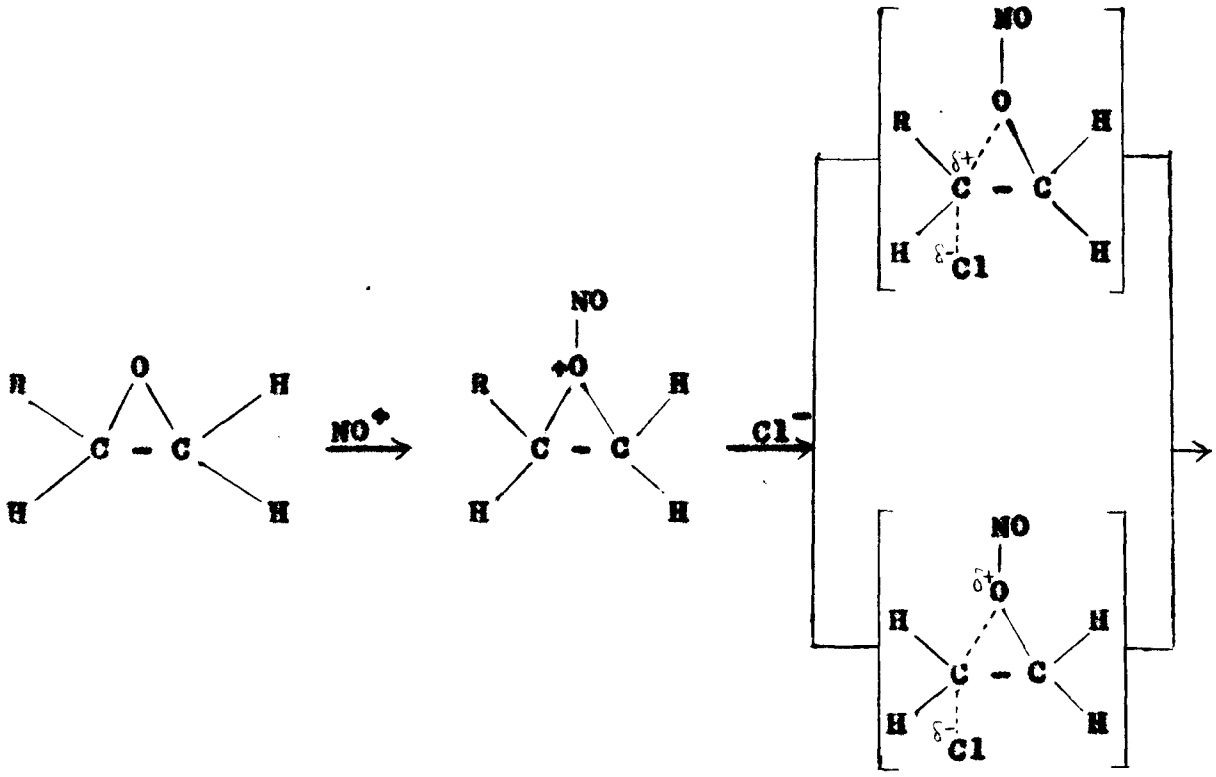
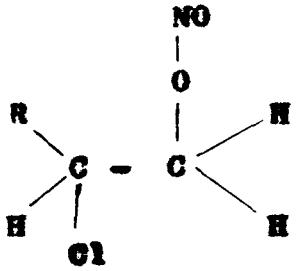


Fig.9. NMR spectrum of 10-chloro-11-nitritoundecanoic acid (XXI)

The exclusive formation of 10-chloro-11-nitrito isomer is in conformity with the reported ring opening reactions of terminal epoxy compounds. A reasonable mechanism for the formation of compound (XXVI) from compound (XXV) is as follows:





$R = -(CH_2)_8CO_2H$

EXPERIMENTAL PROCEDURE

All melting points were observed on a Kofler apparatus and are uncorrected. Infrared (IR) spectra were obtained with a Perkin Elmer 621 spectrophotometer. The abbreviations w, m and str stand for weak, medium and stretching respectively. Ultraviolet (UV) spectra were determined with a Beckman DK-2A spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian A60 NMR spectrometer. Chemical shifts are reported as τ (ppm) relative to tetramethyl silane (TMS). The samples were run as 10% solution in $CDCl_3/CCl_4$. The abbreviations s, d, t, q, m, um, mc and br denote singlet, doublet, triplet, quartet, multiplet, unresolved multiplet, multiplet centred at and broad respectively. Mass spectra (MS) were measured with a Varian MAT-311 (A) mass spectrometer. Microanalyses were performed by Instrumentation Centre, Chemical Laboratories, Aligarh Muslim University, Aligarh. Abbreviations Anal. and Calcd stand for analysis and calculated respectively. Thin layer chromatographic (TLC) plates were coated with silica gel G, and a mixture of petroleum ether-ether-acetic acid (90:20:1, V/V/V) was used as developing solvent. The spots were visualized by charring after spraying with a 20% aqueous solution of perchloric acid. Petroleum ether refers to a fraction of bp 40-60°.

Preparation of nitrosyl chloride *in situ*

The nitrosyl chloride was generated *in situ* by the action of hydrochloric acid on *iso*-amyl nitrite[3]. *Iso*-amyl nitrite was prepared as under:

In a 3 l three-necked round-bottomed flask, fitted with a mechanical stirrer, a separating funnel extending to the bottom of the flask, and a thermometer, were placed 380 g

(5.5 moles) of C.P. $NaNO_2$ and 1.5 l of water. The flask was surrounded by an ice-salt mixture, and the solution was stirred until the temperature falls to 0°. A mixture of 100 ml of water, 136 ml (250 g, 2.5 moles) of conc. H_2SO_4 (Sp. gr. 1.84), and 440 g(5 moles) of commercial iso-amyl alcohol was cooled to 0° and by means of the separating funnel was introduced slowly beneath the surface of the nitrite solution, with stirring. The alcohol solution was added slowly enough so that practically no gases evolved, and temperature was kept at ± 1°. This required for $t\frac{1}{2}$ - 2 hr.

The resulting mixture was allowed to stand in the ice-salt bath until it separated into two layers, and the liquid were decanted from the Na_2SO_4 into a separating funnel. The lower aqueous layer was removed and the iso-amyl nitrite layer washed twice with 50 ml portions of solution containing 2 g $NaHCO_3$ and 25 g of NaCl in 100 ml of water. After drying over 20 g of anhydrous Na_2SO_4, the yield of practically pure iso-amyl nitrite amounted to 81-85% of the theoretical amount.

Nitrosochlorination of methyl oleate

Preparation of methyl oleate (VII)

Pure oleic acid (10.0 g) was dissolved in anhydrous methanol (100 ml) containing catalytic amount of sulphuric acid and refluxed for 1.5 hr. The mixture was then diluted with

water and extracted with ether. The ethereal extract was dried over anhydrous sodium sulphate. Evaporation of ether yielded methyl oleate (VII) as a colourless oil (9.8 g).

Reaction of methyl oleate with approximately stoichiometric quantity of NOCl

A mixture of 3 g (0.01 mole) of methyl oleate and 1.5 g (0.012 mole) of iso-amyl nitrite in 50 ml of methylene chloride was cooled to about 0° in an ice-salt bath. 1.7 ml of conc. HCl was added dropwise with stirring in 30 min. Stirring was continued at ice bath temperature for 1.5 hr. A deep blue coloured solution was obtained. The reaction mixture was washed with water dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The generated iso-amyl alcohol was removed by partitioning between 70% methanol and petrol (1:1). The petrol fraction was dried over anhydrous Na_2SO_4 and the products obtained after evaporation of the solvent were chromatographed over a column of silica gel (30 g). Elution with petroleum ether-ether (95:2, V/V) gave Fraction 1. This fraction was obtained in major amount as a blue liquid. The spectral and combustion data of this fraction are tabulated below:

Anal. Calcd for $C_{19}H_{36}NO_3Cl$: C, 63.04; H, 10.02; N, 3.84. Found: C, 63.12; H, 10.05; N, 3.85.

IR (neat): 3450 (w, OH), 1730 (ester $-\overset{O}{\overset{\|}{C}}-$), 1640 (w, C=N), 1570 (N=O), 1110 (C-N), 710 (C-Cl) cm^{-1}.

NMR (CCl_4): τ 2.52 (=NO<u>H</u>, D_2O exchangeable), 6.12 (1H, -C<u>H</u>Cl-), 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OC\underline{H}_3$), 6.65 [1H, -C<u>H</u> (NO)-] 7.76 (2H, methylene protons α to ester group), 8.38 (2H, methylene protons α to -CHCl-), 8.65 (br s, shielded chain methylene protons), and 9.12 (distorted t, 3H, terminal methyl protons).

Subsequent elution with petroleum ether-ether (95:5, V/V) gave Fraction 2. This fraction was obtained in minor amount (Rf 0.3). The combustion and spectral data are given below:

<u>Anal</u>. Calcd for $C_{19}H_{36}NO_3Cl$: C, 63.04; H, 10.02; N, 3.84. Found: C, 63.10; H, 10.01; N, 3.96.

IR (neat): 3450 (OH), 1730 (ester $-\overset{O}{\overset{\|}{C}}-$), 1640 (m, C=N), 720 (C-Cl) cm^{-1}.

NMR ($CDCl_3$): τ 2.65 (1H, =NO<u>H</u>), 6.1 (1H, -C<u>H</u>Cl-), 6.35 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OC\underline{H}_3$), 7.76 (2H, methylene protons α to ester group), 8.65 (br s, shielded chain methylene protons) and 9.12 (distorted t, terminal methyl protons).

Treatment of methyl oleate with excess of NOCl (in situ)

The methyl oleate (VII) on treatment with excess of NOCl (iso-amyl nitrite + HCl) produced a compound (X) in addition to compounds (VIII) and (IX). The reaction product was worked up as described earlier. The compound (X), having R_f value (0.5) higher than oxime (IX), was characterized as methyl 9(10)-chloro-10(9)-nitrosostearate. The spectral and combustion data of compound (X) are given below:

Anal. Calcd for $C_{19}H_{35}N_2O_4Cl$: C, 58.37; H, 9.02; N, 7.19. Found: C, 58.39; H, 8.98; N, 7.18.

IR (neat): 1730 (s, ester $-\overset{O}{\overset{\|}{C}}-$), 1640 (m, C=N), 1550 and 1360 (NO_2), 710 (C-Cl) cm^{-1}.

NMR (CCl_4): τ 5.96 (1H, $-C\underline{H}Cl-$), 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-O\underline{CH}_3$), 7.38 [t, 2H, $-C\underline{H}_2-C(=N.NO_2)-$], 7.76 (t, 2H, $-C\underline{H}_2-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.67 (br s, shielded methylenes), 9.12 (distorted t, 3H, terminal methyl).

Nitrosochlorination of methyl 10-undecenoate (XI)

Preparation of methyl 10-undecenoate (XI)

Methyl 10-undecenoate (XI) was prepared by refluxing the acid (10.0 g) with absolute methanol (100 ml) and catalytic amount of sulphuric acid as described earlier.

Nitrosochlorination

To the solution of methyl 10-undecenoate (XI, 2.3 g) in 100 ml methylene chloride was added 2.0 g of iso-amyl nitrite and cooled at about 0° in an ice-salt bath. 2.5 ml of conc. HCl was added dropwise with stirring in 30 min. Stirring was continued at ice-salt bath temperature for 1.5 hr. After the usual work-up the products showed the presence of four components on TLC and were chromatographed over a column of silica gel (40 g). Only three components were isolated and characterized in pure form.

Elution with petroleum ether gave a blue coloured liquid which was not analyzed further on account of being unstable.

Subsequent elution with petroleum ether–ether (90:5, V/V) gave methyl 10-chloro-11-nitriminoundecanoate (XV, 0.4 g) as a green oil.

Anal. Calcd for $C_{12}H_{21}N_2O_4Cl$: C, 49.23; H, 7.23; N, 9.58. Found: C, 49.27; H, 7.22; N, 9.58.

IR (neat): 1730 (ester $-\overset{O}{\overset{\|}{C}}-$), 1630 (C=N), 1550 ($NO_2$) and 720 (C–Cl) cm^{-1}.

NMR (CCl_4): τ 3.94 (1H, $-\underline{CH}=N.NO_2$), 5.4 (m, 1H, $-\underline{CH}Cl-$), 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-O\underline{CH_3}$), 7.76 (2H, $-\underline{CH_2}-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.68 (br s, shielded methylenes). Subsequent elution with a mixture of petroleum ether–ether (90:10, V/V) gave methyl 10-chloro-11-oximinoundecenoate (XIV, 0.3 g, mp 42°).

Anal. Calcd for $C_{12}H_{22}O_3NCl$: C, 54.64; H, 8.40; N, 5.31.
Found: C, 54.62; H, 8.49; N, 5.40.
IR (In Nujol): 3300 (OH), 1730 (ester $-\overset{O}{\overset{\|}{C}}-$), 1680 (C=N), 720 (C-Cl) cm^{-1}.
NMR ($CDCl_3$): τ 2.6 (1H, =NOH), 3.6 (1H, $-CH=NOH$), 5.66 (1H, $-CHCl-$), 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 7.76 (2H, CH_2 α to the ester $-\overset{O}{\overset{\|}{C}}-$ group), 8.65 (br s, chain-CH_2-).

Further support to the structure of compound (XIV) was achieved from the analysis of the corresponding carbonyl compound (XVI) obtained by the deoximation of the compound (XIV).

Deoximation of compound (XIV) by levulinic acid and HCl[110]

200 mg of the oxime was mixed with 30 parts of a solution of 9 volumes of levulinic acid and one volume of 1N HCl. This mixture was placed in a erlenmeyer flask and stirred at room temperature for 3 hr. If the oxime was not immediately soluble, it gradually dissolved over the course of the 3 hr. The solution was then diluted with water, extracted with methylene chloride, and the extracts washed free of levulinic acid with bicarbonate solution. The methylene chloride was removed and the carbonyl compound recovered by chromatography.

IR (Neat): 2910, 2840, 1730 (ester and aldehyde carbonyls), 1450 1370, 710 (C-Cl) cm^{-1}.

Subsequent elution with a mixture of petroleum ether-ether (60:40, V/V) gave a dimer of methyl 10-chloro-11-nitroso-undecanoate (XIII, 0.8 g, mp 95°).

Anal. Calcd for $(C_{12}H_{22}O_3NCl)_2$: C, 54.64; H, 8.40; N, 5.31. Found: C, 54.68; H, 8.38; N, 5.38.

IR (In Nujol): 2910, 2940, 1725 (ester $-\overset{O}{\overset{\|}{C}}-$), 1450, 1370, 1270, 1205, 1180, 1160, 710 (C-Cl) cm^{-1}.

NMR ($CDCl_3$): 5.42 (6H, $-\underline{CH}Cl-$ and $-\underline{CH_2}-\overset{O^-}{\overset{|}{N^+}}=$), 6.34 (s, 6H, $-\overset{O}{\overset{\|}{C}}-O\underline{CH_3}$), 7.77 (protons α to the ester $-\overset{O}{\overset{\|}{C}}-$ group), 8.67 (br s, chain methylenes).

Preparation of α,β-Unsaturated fatty acid

The decen-*trans*-2-oic acid was prepared from decenoic acid by the method of Palameta and Prostenik[114].

General procedure

To a well stirred mixture of the saturated acid (25 g) and red phosphorous (1.15 g), dry bromine (12.5 ml) was added dropwise at 90° in a period of 7 hr. The mixture was vigorously stirred during the addition of bromine by using a mercury sealed stirrer. Heating was continued for 24 hr and the cooled solution was poured into cold water and left overnight. The solid product was filtered, extracted with ether, washed with 10% aqueous sodium sulphite solution, then with distilled water and dried over anhydrous sodium sulphate. The 2-bromo acid obtained after evaporation of the ether was heated under reflux with powdered potassium iodide (24 g) in 95% ethanol (175 ml) for 6 hr. To the cooled solution potassium hydroxide (16 g) was added and the mixture was refluxed for another 4 hr. Most of the alcohol was

removed under reduced pressure and the residue diluted with water, acidified with dilute hydrochloric acid, and extracted with ether. The combined ether extracts were washed with water and dried over anhydrous sodium sulphate. After evaporation of the solvent, a mixture of α,β-unsaturated and their co-products, i.e., 2-hydroxy and 2-ethoxy acids were obtained.

The 2-hydroxy acid was separated from α,β-unsaturated acid as a copper chelate by treatment with cupric acetate in ethanol and acetic acid. The remaining two components obtained after removal of 2-hydroxyalkanoic acid were fractionated by silica gel (BDH, 60-120 mesh) column chromatography to afford the individual components. α,β-Unsaturated acid, isolated by elution with petroleum ether-ether (95:5, V/V, yield 80%), was further purified by crystallisation from petroleum ether-ethanol (75:25, V/V)(mp 69°, lit.[115] mp 68.5-69°). The structure of α,β-unsaturated acid was established by elemental and spectral analyses of its methyl ester (XVII) prepared by refluxing the acid (5 g) with absolute methanol (75 ml) and catalytic amount of sulphuric acid as described earlier. The spectral and combustion data of α,β-unsaturated acid water are tabulated below

Methyl docos-trans-2-enoate (XVII)

Anal. Calcd for $C_{23}H_{44}O_2$: C, 78.31; H, 13.57.
Found: C, 78.38; H, 12.54.
IR (CCl_4: 1730 (C=C-COOCH$_3$), 1640 (C=C), and 970 (trans olefin)cm^{-1}
NMR (CCl_4): τ 3.1 d,d (J=15 and 5 Hz; 1H, β to ester carbonyl), 4.0 d (J=15 Hz with a small long range coupling, trans olefinic

proton, 1H, α to ester carbonyl), 6.30 (s, 3H $-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.7 (br s, chain $-CH_2-$) and 9.12 (distorted t, 3H, terminal $-CH_3$).

Nitrosochlorination of methyl docos-*trans*-2-enoate (XVII)

To the solution of methyl docos-*trans*-2-enoate (2.0 g) in 100 ml methylene chloride was added 2.0 g of *iso*-amyl nitrite and cooled to about 0° in an ice-salt bath. 2.5 ml of conc. HCl was added dropwise with constant stirring in 30 min. The reaction flask was kept in a refrigerator at 0-5° for about a month. The reaction mixture was worked up as usual. The reaction product showed the presence of three components on analytical TLC. The major component corresponded to starting material. Column chromatographic separation of the products revealed that only about 10% of the compound (XVII) has reacted. The compounds (XVIII) and (XIX) were formed to the extent of 6 and 4% respectively. The structures of compounds (XVIII) and (XIX) were corroborated with the help of microanalysis, IR and NMR.

Elution with petroleum ether gave starting compound (XVII) and subsequent elution with petroleum ether-ether (90:5, V/V) gave compound (XIX).

Anal. Calcd for $C_{23}H_{43}O_4N_2Cl$: C, 61.79; H, 9.69; N, 6.26.
Found: C, 61.74; H, 9.68; N, 6.28.

IR (neat): 1730 (ester $-\overset{O}{\overset{\|}{C}}-$), 1640 (C=N), 1550 and 1380 (NO_2), 710 (C-Cl) cm^{-1}.

NMR (CCl_4): τ 5.9 (t, 1H, $-CHCl-$), 6.36 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.65 (br s, shielded chain methylenes), and 9.1 (distorted t, 3H, terminal methyl).

Subsequent elution with petroleum ether-ether (90:10, V/V) gave compound (XVIII). Combustion and spectral data are as below:

Anal. Calcd for $C_{23}H_{44}O_3NCl$: C, 66.07; H, 10.60; N, 3.34.
Found: C, 66.12; H, 10.61; N, 3.32.

IR (neat): 3300 (OH), 1730 (ester $-\overset{O}{\overset{\|}{C}}-$), 1640 (C=N), 710 (C-Cl) cm^{-1}.

NMR ($CDCl_3$): τ 2.76 (br, 1H, =N-OH), 6.1 (t, 1H, -CHCl-), 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 9.75 (br s, chain methylene protons), and 9.12 (distorted t, 3H, terminal $-CH_3$).

Reaction of nitrosyl chloride with Fatty 1,2-diol

Preparation of Fatty 1,2-diol (XXI)

Pure 2-hydroxyhexadecanoic acid (XX) (4.0 g, 0.0150 mole) was esterified with absolute methanol (50 ml) containing catalytic amount of sulphuric acid by heating under reflux for 4 hr. The reaction product was extracted with ether, washed and dried over anhydrous sodium sulphate. Evaporation of the solvent gave methyl 2-hydroxyhexadecanoate as a white solid, mp 56-56.5°. The ester (3.8 g, 0.0132 mole) in dry ether (90 ml) was added to a well stirred solution of lithium aluminium hydride (3.8 g) in dry ether (90 ml) at room temperature. The stirring was continued for 1 hr and the excess of reagent was decomposed by a mixture of cold ether-ethyl acetate (95:5, V/V) and cold 10% sulphuric acid. The product was extracted with ether. The combined ether

extracts were washed with water and dried over anhydrous sodium sulphate. Evaporation of ether yielded a solid which on crystallisation from ethanol gave pure 1,2-hexadecanediol (3.4 g, XXI), mp 74-75° (lit.[116] mp 73.1-73.6°).

Anal. Calcd. for $C_{16}H_{34}O_2$: C, 74.36; H, 13.26.
Found: C, 74.29; H, 13.28.

IR (KBr): 3440 br (OH), 2910, 1460, 1150, 1070, 990, 975, 880 and 730 (C-O) cm^{-1}.

NMR (CCl_4): τ 6.4 (1H, $-\underset{OH}{\underset{|}{CH}} - \underset{OH}{\underset{|}{CH_2}}$), 6.8 (d like, 2H, $-\underset{OH}{\underset{|}{CH}} - \underset{OH}{\underset{|}{CH_2}}$), 8.7 (br s, chain $-CH_2-$) and 9.15 (distorted t, 3H, terminal $-CH_3$).

Reaction of nitrosyl chloride with 1,2-diol (XXI)

The 1,2-hexadecanediol (XXI, 1.0 g, 0.0038 mole) was treated with NOCl *in situ* (*iso*-amyl alcohol + HCl) in methylene chloride (35 ml) for 6 hr at room temperature. After the usual work-up the product showed three distinct spots on TLC out of which one corresponded to the spot of starting compound (XXI). The product (~0.88 g) was chromatographed over a column of silica gel (15 g) and the elution was carried out with petroleum ether containing increasing amount of ethyl ether. Elution with petroleum ether gave *iso*-amyl 2-nitritohexadecanoate (XXII, 0.21 g).

Anal. Calcd for $C_{21}H_{41}O_4N$: C, 67.88; H, 11.12; N, 3.76.
Found: C, 67.85; H, 11.11; N, 3.78.

IR (neat): 1730 (ester $-\overset{O}{\overset{\|}{C}}-$), 1630 ($-O-N=O$) cm^{-1}.

NMR (CCl_4): τ 5.9 (mc, 2H, $-O-\underline{CH}_2$), 8.4 (1H, $-\underline{CH}<$), 8.7 (br s, shielded methylene protons), 9.1 (apparent d, 9H, methyl protons).

Mass: m/e 201 (1.7), 191 (0.8), 189 (0.6), 187 (2.3), 173 (0.4), 157 (4.5), 117 (3.8), 97 (3.3), 96 (3.9), 85 (9.0), 73 (3.1), 72 (6.5), 71 (100.0), 70 (12.0), 69 (9.5), 58 (1.8), 57 (11.4), 56 (5.8), 55 (18.7), 53 (1.7).

Subsequent elution with a mixture of petroleum ether–ether (95:5, V/V) gave iso-amyl 2-hydroxyhexadecanoate (XXIII, 0.32 g).

Anal. Calcd for $C_{21}H_{42}O_3N$: C, 70.73; H, 11.97; N, 3.92.
Found: C, 70.78; H, 11.85; N, 3.94.

IR (neat): 3350 (OH), 1730 (ester $-\overset{O}{\overset{\|}{C}}-$) cm^{-1}.

NMR ($CDCl_3$): τ 5.9 (mc, 3H, $-\underline{CH}-OH$ and $-O-\underline{CH}_2-$), 7.3 (1H, $CHO\underline{H}$, D_2O exchangeable), 8.4 (1H, $-\underline{CH}<^{CH_3}_{CH_3}$), 8.65 (br s, shielded chain methylenes), 9.06 (apparent d, 9H, methyls).

Mass: 327 (0.9), 313 (1.2), 295 (0.7), 294 (0.7), 286 (0.9), 294 (6.7), 225 (7.0), 174 (19.8), 133 (7.1), 132 (100.0), 116 (4.4), 113 (4.9), 111 (5.6), 109 (5.0), 104 (4.2), 100 (4.6), 99 (6.6), 97 (10.2), 95 (8.6), 90 (47.6), 85 (11.6), 84 (4.3),

93 (12.5), 82 (6.4), 81 (12.2), 74 (4.1), 71 (18.2), 70 (5.4), 69 (17.7), 68 (4.9), 67 (9.3), 57 (34.9), 56 (8.9), 55 (27.6), 54 (4.9).

Subsequent elution with petroleum ether–ether (80:20, V/V) gave starting material (XXI, 0.25 g) as a solid mp and mmp 74–75°.

Reaction of NOCl with 10,11-epoxyundecanoic acid (XXV)

Preparation of 10,11-epoxyundecanoic acid[111]

The 10-undecenoic acid (1.3 g) reacted with m-chloroperbenzoic acid (1.2 g) in chloroform (150 ml) at room temperature for 3–4 hr. The epoxy acid, recovered by ether extraction in almost quantitative yield, was purified by preparative TLC using silica (1 mm) and petroleum ether–ether (80:20, V/V) as developing solvent.

Preparation of nitrosyl chloride and its reaction to 10,11-epoxyundecanoic acid (XXV)

Nitrosyl chloride was prepared by the action of sodium nitrite and HCl as described by Morton and Wilcox[110]. This procedure uses a modification of the previously reported methods. The process is advantageous in that there are no intermediates to purify and the crude nitrosyl chloride contains only oxides of nitrogen. The gas was purified by passing through tubes

containing sodium nitrite (to absorb hydrogen chloride), potassium chloride which has been moistened with an amount of water equivalent to 2.4% of its dry weight (to absorb nitrogen dioxide), and anhydrous calcium chloride. The NOCl gas thus purified, was directly used for the reaction as under:

A solution of 1 g of 10,11-epoxyundecanoic acid in methylene chloride was taken in a round-bottomed flask and cooled to about 0° in an ice-salt bath. The nitrosyl chloride gas was passed slowly into the reaction mixture with constant stirring at 0° for 15 min. After the gas discontinued the stirring was continued for 1 hr more at ice-salt bath temperature. The reaction mixture showed a single spot on TLC having R_f value slightly lower than the epoxy compound showing thereby a quantitative yield of the product (XXVI, 0.9 g).

Anal. Calcd for $C_{11}H_{20}O_4NCl$: C, 49.71; H, 7.58; N, 5.27. Found: C, 49.75; H, 7.57; N, 5.26.

IR (neat): 1710 (Acid $-\overset{O}{\overset{\|}{C}}-$), 1630 ($-O-N=O$), 720 (C-Cl) cm^{-1}.

NMR (CCl_4): τ 2.47 (s, 1H, $-\overset{O}{\overset{\|}{C}}-O\underline{H}$), 5.9 (1H, $-C\underline{H}Cl-$), 6.4 (d, 2H, $-O-C\underline{H}_2-$), 7.7 (2H, protons α to $-CO_2H$ group), and 8.65 (br s, shielded methylene protons).

Compound (XXVII) was obtained by the dechlorination of compound (XXVI) using method of Jungermann and Speerri[117] as described below:

To the solution of the compound (XXVI, 0.25 g) in 30 ml of glacial acetic acid, zinc amalgam (1.0 g) was added. The mixture was refluxed for 6 hr. The reaction mixture was extracted with ether after dilution with water. The ethereal extract was dried over anhydrous sodium sulphate and evaporated to dryness. The product was not found pure as shown by analytical TLC. The NMR of impure sample, however, showed all the signals displayed by compound (XXVI) except the signal at τ 5.9.

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